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MYCOLOGIA

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VOL. XL MAY–JUNE, 1948 NO. 3

THE DEVELOPMENTAL PATTERN WITHIN THE GENUS PLEOSPORA RAB.

L. E. WEHMEYER *

(WITH 56 FIGURES)

The taxonomic situation in the genus *Pleospora* is similar to that to be found in many other large genera of the fungi, or for that matter any group of organisms which, as a result of a successful adaptation to their habitat, is in an active state of evolution. In a previous study (**5**) of this genus in northwestern Wyoming, it was pointed out that if a sufficiently large number of collections were examined and tabulated according to some important character such as spore size, there would be found a long series of collections with overlapping variations, within which it would be impossible to detect any natural specific lines of distinction. Since this earlier study loans kindly made by a number of herbaria have made it possible to study some 500 collections of *Pleospora,* which have given a far more extensive, though yet far from complete, picture of the natural population of this genus.

The same general situation still obtains. Although occasional collections or groups of collections stand out as distinct, the great majority fall into larger groupings showing the overlapping variations mentioned for any one of a number of important characters. Although an analysis of these variable characters shows that they vary independently of one another, yet there are certain general

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correlations which enable one to plot the general pattern of evolutionary tendencies within the natural population.

Once this pattern is determined, all collections can be arranged in series accordingly and the range of variation in nature more nearly determined. The circumscription of species, following such a policy, is in the main the segregation of sections of these series or species-complexes upon the basis of a loose correlation of the factor variants or often upon a purely arbitrary basis. It soon becomes evident that in either case there are bound to be intermediates or modifications, so that extremes of any one species will approach or overlap those of neighboring ones.

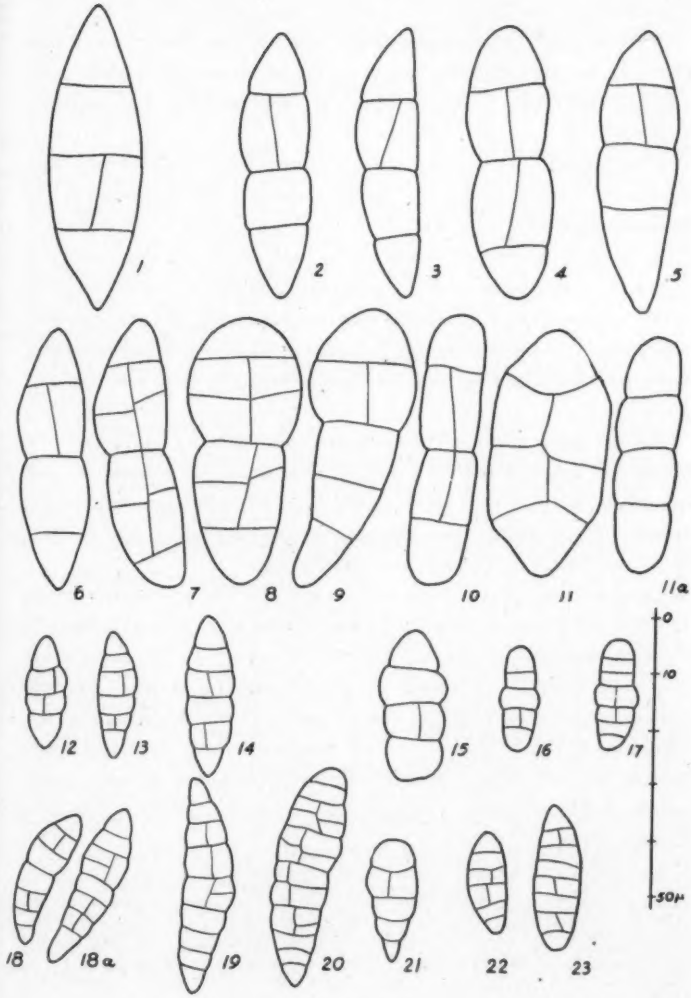
In the past, most species within the genus have been described upon the basis of one or at most a few collections. Such collections at the two extremes of any series or species-complex appear quite distinct. As other collections intermediate within the same series are found to differ in certain factors, they are again described as new. Such a backward approach to the problem ultimately leads to a confusion of overlapping species, which necessitates the description of nearly every collection as a new species.

It is not the purpose of the present paper to delimit species nor to assign binomials, but rather to analyze the variable factors which are of diagnostic importance and to plot the developmental pattern within the genus.

ASCOSPORES. As might be expected, the characters of the ascospore, its form, size, septation, and color are the most useful in determining relationships.

Form: The muriform spore of *Pleospora* has no doubt been derived from that of *Leptosphaeria* by the insertion of vertical septa in some of the cells. The basic simplest spore-form is the fusoid, three-septate, straight, symmetric, non-constricted one shown in figure 1. An analysis of the many spore forms within the genus shows that they are the result of various combinations of the following four modifications of this basic form. The descriptive terms given are those used for these modifications.

1. An increase in the area of the side walls of the spore after the laying down of the transverse septa results in constrictions at the septa. Such constrictions are usually greatest at the first septum



FIGS. 1–23. Spores of *Pleospora*.

to be formed (usually the central one). *a.* Non-constricted. *b.* Constricted (FIG. 2).

2. An unequal development of the side walls of the spore, so that one side becomes flattened or concave, whereas the opposite side remains convex. *a.* Equilateral. *b.* Inequilateral (or curved) (FIG. 3).

3. A rounding of the end walls of the spore, resulting in an ellipsoid or oblong shape. *a.* Ends acute. *b.* Ends rounded (or abruptly tapered) (FIG. 4).

4. An unequal development of the two halves of the spore, on opposite sides of the primary septum, resulting in an asymmetric spore. In such cases the upper portion is usually shorter, broader, and commonly more rounded, whereas the lower portion is longer, narrower, and often tapered. *a.* Symmetric. *b.* Asymmetric (FIG. 5).

A fifth spore form is the clathrate type (FIGS. 11 and 11a), in which the spore is flattened and vertical septa are formed in only one plane, so that they show in the face, but not edge view of the spore. These species have been placed in a separate genus *Clathrospora,* and do seem to form a distinct group.

Combinations of the above factors give the six main spore forms found in the genus and referred to as follows: The *fusoid type* (FIG. 6) characteristic of the leptosphaeroid series. The *fusoid-ellipsoid type* (FIG. 7) characteristic of the *vulgaris* series. The *oblong-ellipsoid type* (FIG. 8) characteristic of the *herbarum* series. The *clavate type* (FIG. 9) often associated with asymmetrically septate spores. The *oblong type* (FIG. 10) found in only a few species. The *flattened clathrate type* (FIGS. 11 and 11a) found in *Clathrospora.*

Septation: The basic spore of the genus is three-septate. The central septum is usually laid down first, but soon is followed by two secondary transverse septa, one on each side. Beyond this three-septate condition there are several modes of septation, as follows.

In the leptosphaeroid series the transverse septa are laid down first and secondary septa are then formed in the end cells giving a five-septate and then a seven-septate spore. This septation of the end cells often leaves the central cells somewhat larger, and one or

both of them may become somewhat swollen, as is commonly the case in *Leptosphaeria*. In this series the vertical septa are rather tardily formed and many, or even all, of the cells may be without them.

In the *vulgaris* and *herbarum* series the secondary septa first appear in the central cells and the vertical septa are usually formed first. In such a vertically septate central cell the transverse septa formed on each side of this vertical septum are often laid down at different times, at different levels, or at different angles (FIGS. 7–8), giving them a characteristic irregular arrangement. The seven-septate spore arises by septation of the two end cells, which is also commonly irregular in that the vertical wall splits into a "Y" shaped septum or shows a transverse septum on only one side. Further septation is variable and will be discussed under these series.

The asymmetric type of septation results from the insertion of extra transverse walls, nearly always in the lower end of the spore. This type of septation occurs in all types of spores and does not constitute a separate series.

Color: The color of the spore in *Pleospora* varies from a pale honey yellow through darker yellow-brown to a dark red-brown. This of course ignores the hyaline spores of *Catharinia, Pleosphaerulina,* etc. The simpler leptosphaeroid spores are nearly all yellow-brown. The red-brown color appears to be more or less correlated with growth at higher altitudes or latitudes. Many species show this complete color range, unless color itself is used for species separation. The color also darkens with maturity, and a single collection may show a wide range of color.

PERITHECIA. The perithecium of *Pleospora* has a dothideaceous (pseudosphaeriaceous) type of development. Consequently the cavity arises as a differentiated central area within a stroma, and the wall is in reality the remainder of the sterile stromatic tissue. Great emphasis has been placed in the past upon the comparative thickness of this wall, and such genera as *Scleroplea* have been based upon it. Certain species, as *P. herbarum,* are characterized by a thick wall, but this thickness depends upon environmental conditions of growth and is only of secondary importance even for species differentiation. The same applies to perithecial size which

may vary greatly within one species under varying conditions, but in other cases, in correlation with other characters, may indicate distinct groupings.

The formation of setae about the ostiole of the perithecium has also been used for generic separation (*Pyrenophora*). Many species of *Pleospora* will show a few, brown, creeping hyphae growing out from the perithecium, particularly at the base. All degrees of tomentosity from this to a dense outgrowth of basal tortuous brown hyphae or a woolly mass of hyphae covering the entire perithecium can be found. In these latter cases there is a tendency for the hyphae on the surface of the perithecium to become stiff, upright and pointed, and to penetrate the overlying epidermis. At other times these upright setae are numerous and definite. This tendency for the formation of setae is also more pronounced at high altitudes and latitudes, and also where the overlying host tissues are fragile or soon thrown off. If arranged according to spore form, septation, size, and color, these setose forms comprise a series almost directly parallel with forms having smooth perithecia. The setose condition seems to be of no more than specific or even varietal or habitat rank. A similar opinion has recently been put forward by Petrak (2, p. 446).

ASCI. The asci of the genus vary in shape from almost globose or saccate, through broadly clavate to cylindric. They nearly all have a thickened inner gelatinous wall, often more pronounced above, and an expanded, disc-like or "claw-like," basal attachment. They vary in size and shape with maturity, becoming narrower and longer as the spores are ejected. Although often helpful as a secondary character, they are not of prime importance in the delimitation of species.

HABITAT. There are several modifications which seem to be correlated with habitat. Collections on leaves, petioles, sepals, or minute stems often show small perithecia (200 μ or less in diameter), but similar small perithecia may also occur on herbaceous stems, and larger perithecia on leaves. This appears to be an effect of the habitat rather than an adapted variation of the fungus.

A more intriguing habitat group, but one more difficult to fix, is the one on woody stems. On wood and bark the perithecia tend to be larger with thick stromatic walls and often clustered or con-

fluent, and the asci are more numerous, elongate, cylindric and with uniseriate spores. Although these characters are not definitely correlated with this habitat, most species with a wide variety of spore size, form, and septation show one or more of these characters. These characters are also found in the genus *Teichospora*, which is probably derived from this group.

All of the characters discussed may vary independently of one another, but certain characters or combinations often persist through a series of changes in other characters. The important question is how much variation may occur within an individual mycelium or a species. If one represents the four contrasting pairs of characters of spore form by the indicated letters *a* and *b*, the number of septa by a numeral and the color by Y (yellow), B (brown) or R (red-brown), a formula can be written for any given spore. Such a spore as in figure 1, for instance, would be *a,a,a, a*,3,Y, or for figure 8 perhaps *b,a,b,b*,5,B. With such formulae as a mechanical aid, it soon becomes apparent that the spores of any collection, any perithecium, or even a single ascus, cannot all be represented by the same formula. Different collections, apparently of the same species, will show different percentages of spores having certain formulae. Again the factors of form, color, and septation change with maturity of the spore. In any mature collection, however, the spores do not go beyond a certain stage of development. The most mature spores, therefore, are the diagnostic ones.

Theoretically, an individual is the mycelium, perithecia, conidia, and ascospores from a single spore. In nature, however, such a derivation is next to impossible to determine. Most infections are probably from more than one ascospore or conidium, and it is a common observation to find several species of *Pleospora* mixed upon one stem or leaf. Even in the comparison of spores of a single perithecium or ascus, the possibility of heterokaryosis or hybridization must be taken into consideration. Until the genetic mechanism of perithecial and ascus formation in the genus is known and genetical studies have been made, therefore, the behavior of these factors cannot be known and specific variation and segregation must be based upon personal judgment from morphologic evidence.

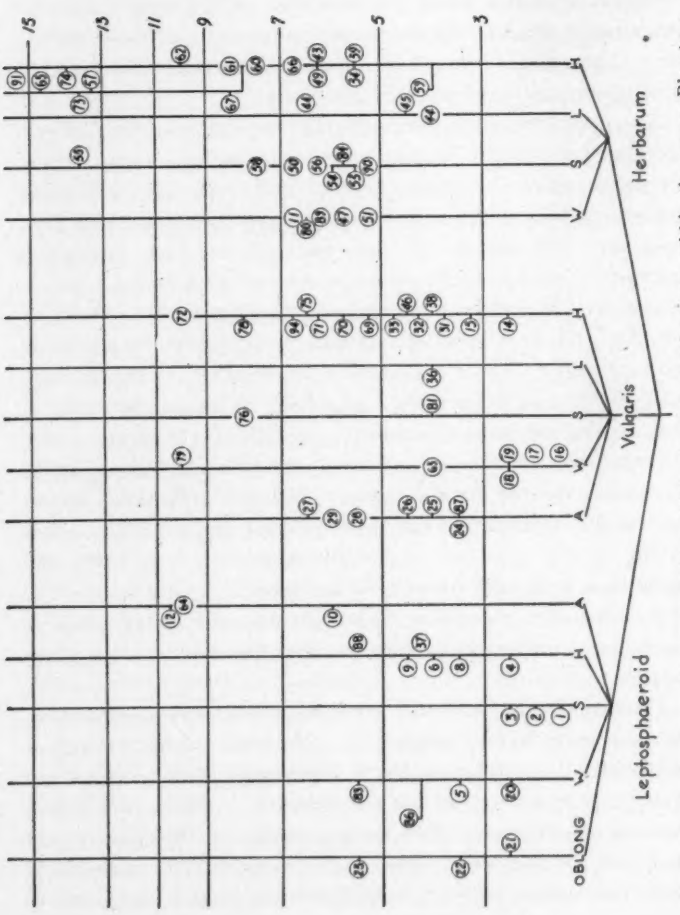


CHART I. A diagrammatic representation of the developmental pattern within the genus *Pleospora*. Explanation in text.

Chart I is an attempt to plot the natural arrangement of the collections studied. The three main branches of the chart represent the three main series of development within the genus, in each of which a typical spore form is retained. These are indicated as the *leptosphaeroid, vulgaris* and *herbarum* series respectively. In each of these series certain groups of species are related upon the basis of other characters. These are represented as secondary branches and designated by letters as follows:

H. The typical forms on herbaceous stems.
L. Leaf inhabiting forms which sometimes are distinct enough to represent species, but in others are not.
W. The wood inhabiting forms which in most cases show the distinctions already enumerated.
S. Those collections with setose (not merely tomentose) perithecia, which usually run parallel with and correspond to species with non-setose perithecia.
A. The collections showing spores with asymmetrical septation appear to have been derived from various types with a symmetric septation, and do not form a distinct series.

The small group with oblong spores, at the extreme left, seem to be related to the leptosphaeroid series. The horizontal lines represent levels of septation, the number of septa at each level being indicated at the right. The numbers enclosed in circles indicate what seem to the writer to be natural species-groups. Inasmuch as these groups of collections do not always coincide to species as now described, or include several described species, they are referred to here by number only. When such species groups have spores which are predominantly yellow-brown, the encircled numbers are placed to the left of the vertical line; if the spores be red-brown, to the right of the line; or if the spores range from yellow- to red-brown, the encircled numbers are placed upon the line.

CHART I. A diagrammatic representation of the developmental pattern within the genus *Pleospora*. Explanation in text.

LEPTOSPHAEROID SERIES

This series is derived from the basic fusoid, three-septate, yellow-brown spore. Throughout the series the spores are recognizable in having the following general characters: 1. They are mostly nar-

rowly fusoid, with gradually tapering, acute ends. 2. They are more symmetrical, equilateral and less strongly constricted than in the other two series. 3. The color is always yellow-brown, and often pale yellow-brown. 4. The septation is distinctive and of the type previously described for this series.

Figures 12 to 20, which are spores of this series, are illustrative of these characters. These figures of spores, and the remaining ones (except for the first 11 figures), are camera lucida drawings made to scale and chosen as typical of each particular collection.

The spore in figure 12 represents species No. 4 of the chart, which has three-septate spores in smooth perithecia commonly found on *Papaver,* and is probably *P. pellita* (Fr.) Rab. and *P. papaveracea* (Not.) Sacc. Similar spores found in tomentose perithecia with some setae probably belong to *P. calvescens* (Fr.) Tul. (species No. 3). *Pyrenophora hyphasmatis* E. & E. (No. 1), with tomentose perithecia, and *Pleospora delicatula* Vestergren (No. 2), with setose perithecia, both have even simpler three-septate spores which often entirely lack vertical walls in the cells. The insertion of two secondary septa in the end cells gives the five-septate spore of this series as shown in figure 13, taken from an unidentified collection on *Phleum* from Sweden, and is characteristic of species No. 6, which has small perithecia, is commonly found on grasses, and probably corresponds to *P. vagans* Niessl. The spores of this group range from 18 to 30 μ in length, the largest spores being found in type material of *P. fuegiana* Speg. and *P. Forsteri* Speg. from Tierra del Fuego. Figure 14 is such a large spore found on the type of *Leptosphaeria Yerbae* Speg. It is not the *Leptosphaeria* described by Spegazzini, but a *Pleospora* of the *vagans* type but with large spores, large perithecia, and on woody stems (No. 83). Other woody forms found in this series are No. 20, a collection of *P. Thuemeniana* Sacc. on *Agave,* with three-septate spores, and No. 5, a collection of *P. parvula* Berl., on *Clematis* with 3–5-septate spores. No. 86 (FIG. 21) is an undetermined collection on *Sorbus* from Sweden having asymmetrically septate spores with four to six septa.

This tendency toward asymmetric septation by the formation of extra septa in the lower end cell of the spore also occurs in herbaceous forms. No. 10 (FIGS. 18, 18a) includes type material of *P.*

intermedia Speg., and *P. Niessleana* Kze., and collections of *P. dura* Niessl which have spores with six to eight septa. No. 12 (FIG. 19) is a collection of *P. dura* which shows a further insertion of septa to give spores which have eight to ten septa.

No. 64 (FIG. 20) is an example of one of the troublesome intermediate forms. It is a collection of *P. abscondita* Sacc. & Roum. on *Phragmites* and shows asymmetrically septate spores with three to four septa above and four to five, rarely six, below the primary septum and therefore is eight to ten, rarely eleven septate. Furthermore there are secondary septa often laid down in the manner described for the *vulgaris* series. The figure shows an extreme case of this. Such septation is also rarely found in Nos. 10 and 12 (FIGS. 18a, 19). In other words, as the number of septa increases in the leptosphaeroid and *vulgaris* series, the spores approach one another in appearance and the two series are difficult to distinguish.

Nos. 37 and 88 are also rather anomalous species, both occurring on *Galium*. No. 37 (FIG. 22) is an unpublished species, labeled *Pleospora Galii* Romell in the Riksmuseum Herbarium. The spores are five-septate and have the general fusoid-ellipsoid form of the *vulgaris* series. The spores are not constricted, however, and show the leptosphaeroid transverse septation, with vertical septa in only a few central cells. No. 88 (FIG. 23) is an undetermined collection, also in the Riksmuseum Herbarium. The spores are identical in form, but larger and with seven septa. Such intermediate species as these merely demonstrate that the "series" as here outlined are merely generalizations covering the majority of cases, and that other combinations of characters may, and do, exist.

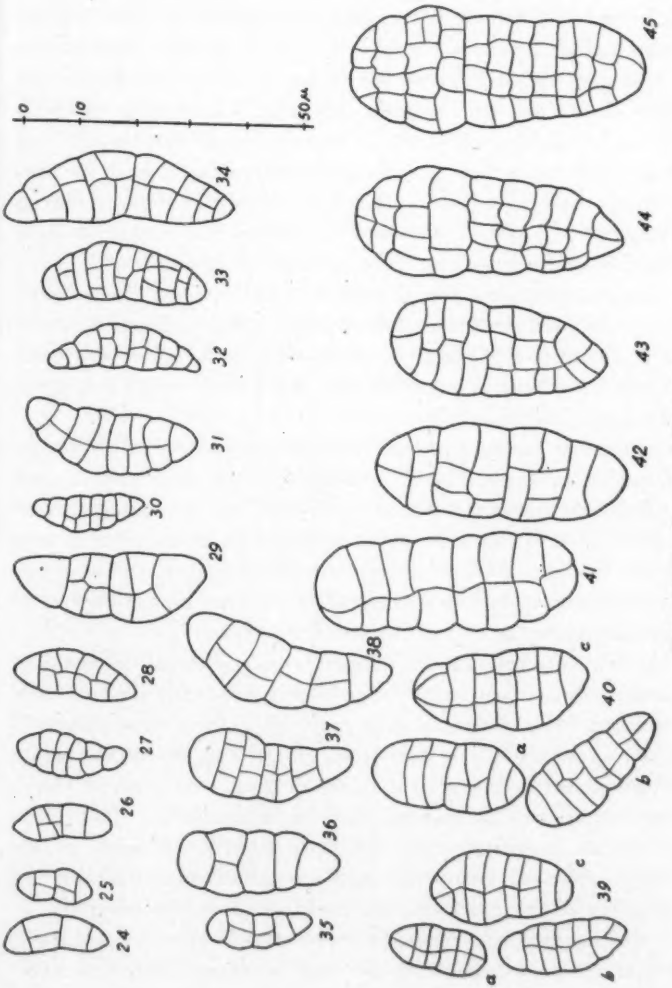
Nos. 15, 16, and 17 are again a small group of species with an oblong spore-form, which are represented as a subdivision of the leptosphaeroid series because of their septation. No. 21 (FIG. 15) is represented by the type of *P. mollis* Starb. These spores are dark red-brown, which is atypical for this series. No. 22 (FIG. 16) has spores with four or five septa (an extra septum in the upper half in the latter case) and agrees with the description and collections of *P. oblongata* Niessl. No. 23 (FIG. 17) has the same spore

form but with six septa. Two collections by Rehm (labeled as a variety) in Riksmuseet show these spores.

VULGARIS SERIES

This series is based upon a somewhat subtle change in spore form, and a type of septation differing from that of the preceding series as already described. The spores have more bluntly tapered or rounded ends, are more commonly inequilateral or curved, and the end cells are never septate. Figures 24 through 38 show spores of this series. It can be derived from the three-septate level, through collections such as are found in No. 14 (FIGS. 24, 25), which show a broadening of the spores and rounding or blunt tapering of the ends. No. 14 includes the type collections of *P. oligostachyae* E. & E. (FIG. 24) and *P. diaporthoides* E. & E. and others, in which the spore form is somewhat variable but always with blunted ends. Some collections of *P. diaporthoides* show some spores with secondary septa of the *vulgaris* type in the central cells. This condition is more commonly found in type material of *P. Boldoae* Speg. (FIG. 26), which is intermediate between No. 14 and No. 33 but is placed in the latter group.

The five-septate condition is attained in this series by the vertical and transverse septation of the two central cells. This stage is represented by Nos. 31, 32, 33 and 38 which comprise a large number of collections with three- to five-septate spores showing many overlapping variations in spore form and size ($14–26 \times 5.5–9\ \mu$). No. 31 represents *P. socialis* which has rather small spores and perithecia formed in heavily blackened areas. No. 32 is *P. infectoria* Fck. the type of which shows similar spores (FIG. 27) in perithecia clustered in linear groups on grass stems. The remaining collections of this sort are placed in No. 33 (FIG. 28) which probably corresponds to *P. vulgaris* Niessl. The three species groups just mentioned all have yellow-brown spores. Collections of this type from western America show slightly larger spores with a dark red-brown color and have been considered a variety (No. 38) of *P. vulgaris*. In this same region, collections with these red-brown spores show a continuation of the range of spore size far beyond that of *P. vulgaris*, reaching $26–35(40) \times 8–14\ \mu$. This larger spore group corresponds to *P. richtophensis* E. & E. (FIG. 29).



FIGS. 24–45. Spores of *Pleospora*.

The next step is the insertion of a transverse septum in each end cell to form a seven-septate spore. In such cases the inner cell so formed may become vertically septate but the terminal cell remains without a vertical septum. Spores of this type are found in a collection labeled *P. rubicunda* Niessl (but not that species), in Riksmuseet (No. 69, FIG. 30) and of *P. Thurgoviana* Wegelin (No. 70, FIG. 31). Nos. 70, 71 and 94 are all very similar and occur on Monocotyledons such as grasses, rushes, *Typha*, etc., inhabiting swampy margins. They differ chiefly in their perithecial configuration. No. 75, *P. tomentosa* Wehm., has similar but dark red-brown spores and densely tomentose perithecia.

A nine-septate condition is reached in this series by the insertion of an additional septum in the end cells. This is shown in spores (FIG. 32) from the type of *P. Alismatis* E. & E. (No. 78), which may be considered as a continuation of the series mentioned above on aquatic Monocotyledons.

In another group of collections found under *P. Anthyllidis* var. *Aconiti* Rehm (FIG. 33), *P. rubicunda* Niessl, etc.(No. 72), and probably belonging to the latter species, the spores reach an eleven-septate condition with five septa on each side of the primary one. These spores are broader, with more rounded ends, and have one to three vertical septa in the central cells. Here four of the central cells may show the *vulgaris* type of septation.

It will be noted that the irregular "*vulgaris* type" of septation occurs only in the two central cells of spores with seven to nine septa, leaving the two or three end cells with a "leptosphaeroid type" of septation. It was also pointed out under the leptosphaeroid series that the asymmetric types with nine to eleven septa might show the *vulgaris* septation in some cells. This makes it difficult to separate these two series, but since nearly all the spores with more than seven septa are rather bluntly rounded and inequilateral or curved, they are placed in the *vulgaris* series.

Although many collections of the *P. vulgaris* type have perithecia which are tomentose, very few have been seen with true setae. No. 81 has spores of the *P. richtophensis* type, but lighter yellow-brown, and setose perithecia, and is an undetermined collection. *P. pleosphaeroides* Wehm. (No. 76, FIG. 34) has nine-septate

spores, as in *P. Alismatis*, but much larger, and perithecia covered with stiff spines.

The woody forms are also rather restricted in this series. The type of *P. atromaculans* Rehm (No. 16), on *Cornus*, has small three-septate spores, small perithecia, and is closely related to No. 14. Nos. 17, 18 and 19 represent the types of *P. carpinicola* E. & E., *P. Juglandis* E. & E. and *P. Shepherdiae* Pk. respectively. They all have large perithecia and three-septate spores, and differ chiefly in slight differences in spore size. These spores, again, differ from such as those of *P. Thuemeniana* of the leptosphaeroid series chiefly in the greater diameter and more rounded ends. No. 79 is *Pyrenophora moravica* Petr., with irregularly seven- to 11-septate spores which are intermediate between Nos. 64 and 72.

The collections of the *vulgaris* series with asymmetrically septate spores are interesting in that they all seem derived from similar forms with symmetric septation by the addition of a septum in the lower end (or perhaps the dropping out of a septum in the upper half). They all show the rounded ends of this series. The type of *P. kansensis* E. &. E. (No. 24) has four-septate yellow-brown spores (FIG. 35) which might be derived from such as are found in No. 14 by the insertion of a septum in the lower end cell. The type collection of *P. infectoria* var. *nigriseda* Rehm (No. 87) has similar four-septate spores, but they are dark red-brown, and many spores are three-septate. Nos. 25 and 26 represent misdetermined collections of *Pyrenophora phaeocomoides* and *Pleospora herbarum* (FIG. 36) respectively with rather dark brown to red-brown four-septate spores, much larger than in No. 87. No. 25 appears to be *P. richtophensis* in which no secondary septum was laid down in the upper half. In fact some perithecia show many spores which are five-septate and show such septation. Nos. 27, 28 and 29 are all six-septate by the addition of a cross-wall in each end of the spore. No. 28 is a collection labeled *P. oligotricha* Niessl, which has rather small yellow-brown six-septate spores, which appear as *vulgaris* spores with an extra septum in the lower half. No. 27 (FIG. 38) represents several collections with larger red-brown spores similarly related to *P. richtophensis*. No. 29 (FIG. 37) includes collections of *P. orbicularis* Auersw. (*P. Berberidis* Rab.) which

have six-septate spores with a characteristic clavate form, the upper portion being strongly rounded and the lower long-tapered.

HERBARUM SERIES

At the five-septate level of the *vulgaris* series certain changes occur in spore form and septation which give rise to the *herbarum* series. This area of transition from the *vulgaris* to the *herbarum* spore type is the most difficult one in the genus because the species here concerned are the most abundant and widely distributed and consequently show all sorts of character combinations and overlapping variations. The result is an evenly graded series of changes in which specific limits must be chosen in a purely arbitrary manner. The changes which occur to give the *herbarum* type of spores may be enumerated as follows:

1. The spore becomes even broader and with more rounded ends, resulting in an oblong-ellipsoid shape.
2. There is a tendency for the spores to be more asymmetric but less inequilateral or curved, becoming straight with a longer, narrower, tapered lower end.
3. Vertical septa are formed in the end cells. These often first appear as branched "Y" shaped septa, thereby also increasing the number of cross septa.

As the transition from *P. vulgaris* (No. 33) or its red-brown variety (No. 38) to the corresponding *P. herbarum* (No. 49) or its var. *occidentalis* (No. 43) is a gradual one with all sorts of variants, it is necessary to choose some arbitrary character to limit *P. vulgaris* and the *vulgaris* series. The presence of vertical septa within the end cell of at least a fair percentage (10–100%) of the spores has been taken as such a criterion.

This large group of collections, with spores five- to seven-septate, 14–28 × 7–12 μ, and always with some spores with vertically septate end cells, contains the type collections of a dozen or more described species, but probably lies nearest to the *P. media* of Niessl, which he states (1) differs from *P. vulgaris* only in the darker color and vertically septate end cells. This group is divided into a yellow-brown spored variety (No. 34) and one with red-brown spores (No. 39) which are somewhat larger. In this group, some

collections will show small, yellow-brown, broadly rounded, five-septate spores with vertically septate end cells (as in FIG. 39a); others will have larger, red-brown, fusoid-ellipsoid, six- or seven-septate spores (39b); large, red-brown, broadly ovoid, seven-septate spores (39c) or many other combinations of these characters.

This series continues in an unbroken fashion, with all possible variants, through the many collections commonly referred to *P. herbarum* (Fr.) Rab. There have been a score or more of species described upon the basis of collections falling within the range of *P. herbarum* as here outlined. Although occasional collections seem to be distinct, no group of collections can be found which appears distinct enough to be called a species. *P. herbarum* is here arbitrarily limited to those species with spores having five to seven, mostly seven, septa, and which measure 22–40 × 9–14 μ. It will be noted that the spore measurements of *P. media* (No. 34 and No. 39) overlap those of this group. However, collections of *P. media* with spores longer than 22 μ will always show some shorter than this, whereas collections of *P. herbarum* with spores shorter than 26 μ will always show some which are longer than this.

The collections of *P. herbarum* on leaves are represented on the chart as No. 48. Many of these collections have small perithecia (100–200 μ), somewhat smaller spores and a high percentage of five-septate spores. On the other hand, such forms also occur upon stems, and some collections on leaves show large perithecia and larger, seven-septate spores. These may, therefore, represent merely a doubtful habitat grouping.

Two varieties, No. 49, with yellow-brown spores, and No. 43, with red-brown spores, are again recognized. Figure 40a is taken from the type of *P. Herniariae* Fck., on leaves. Figure 40b is from Rab. Herb. Myc., Ed. I, No. 547a, and represents the yellow-brown asymmetric spore type common in most European collections as well as in many others, and which are placed on the chart as No. 49. Figure 40c is characteristic of the red-brown type of spore commonly found in the mountain regions of western America and described by the writer as var. *occidentalis* (No. 43). This variety often shows more inequilateral spores.

In the larger spores of *P. herbarum* and throughout the spores of the rest of this series, the number of vertical septa laid down

increases, so that there are often from two to three or more vertical septa visible in face view between any two transverse septa. Such vertical septa usually extend continuously through several or even all cells, but this is not always true by any means.

The upper limit of spore size for *P. herbarum* is arbitrarily set at $40 \times 14\,\mu$. There are, however, many collections with similar spores but of greater size ($30–55 \times 16–20\,\mu$). Many collections show small spores which fall within the *P. herbarum* range, but also spores larger than $40 \times 14\,\mu$. Such collections appear under a number of binomials and are grouped here under No. 66. The type collections of *P. maritima* Rehm (FIG. 42), *P. Jaapiana* Rehm (FIG. 41), *P. Armeriae* (Rab.) Ces. & deNot, and *P. Balsamorrhizae* Tracy & Earle fall in this group. Some of these collections show rather characteristic spore forms but further collections are needed to determine whether these are constant.

The next change in septation, after the seven-septate *P. herbarum* type, is the appearance of irregular septa in the lower end of the spore at angles other than right angles to the spore axis. This results in an irregular fourth septum in the lower end, giving an eight-septate spore (FIG. 43). Such spores were previously referred to *P. stenospora* (**5**), but the type collection of *P. coloradensis* E. & E. also has them. These collections are represented on the chart as No. 60. These spores are red-brown and asymmetric, the lower half always being longer, narrower and somewhat tapered. This asymmetry of both form and septation is characteristic of all the remaining species in this series.

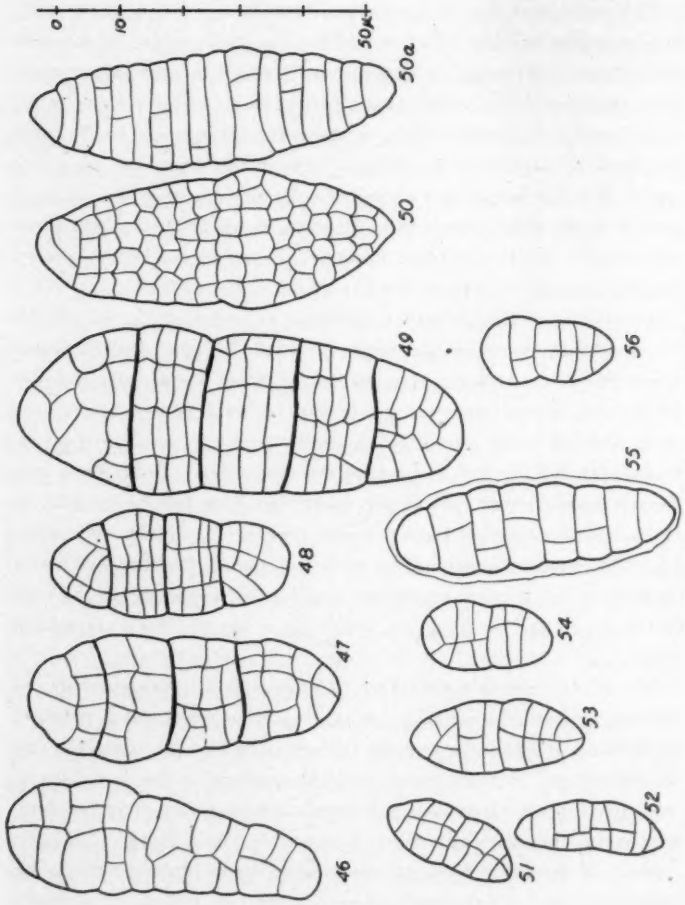
It is necessary here to examine the sequence of formation of the transverse septa. If the first three septa laid down be considered as primary, the five-septate spore (of this series) arises by the formation of secondary septa in the two central cells and the seven-septate spore by the additional septa in the two end cells. In No. 60, an additional eighth septum is irregularly laid down in the lower end giving rise to an asymmetric septation. The next step, found in No. 61 (FIG. 44), is the insertion of one or two regular cross walls in the lower end of the spore, giving one with eight or nine septa. Sometimes the formation of irregular oblique septa (as in No. 60) in either end makes these spores appear ten- to eleven-septate. The writer has collections of this type only from the

Rocky Mountain region. The numerous collections reported by Petrak (2, 3) as *P. chlamydospora* Sacc. on *Astragalus* from the mountainous regions of Iran also seem to belong to this group.

The next change in septation is a distinct one and is seen in *P. montana* Wehm. (No. 62) (FIG. 45). The spores show three septa above and usually six to seven septa below the primary septum. This septation is the result of the formation of tertiary septa in all of the cells in the lower half of a seven-septate spore. In *P. montana* this origin is not so obvious because the septa are regularly laid down, but in another series of collections, shown as a lateral branch to the left of those on herbaceous stems in the *herbarum* series on the chart, the septa are tardily and incompletely formed and this manner of origin is more strikingly shown.

The origin of this irregular septation is found in No. 67, which includes the type collections of *P. laxa* Ell. & Galw. and *P. longispora* Speg. (FIG. 46). The spores of these species are yellow-brown and basically seven-septate, but incomplete, tertiary, cross-septa are commonly found laid down between two neighboring vertical septa but do not extend across the spore. Such septa are usually in the lower half of the spore, but give rise to irregularly eight- to nine-septate spores. The type of *P. pulchra* Kirschst. (No. 73) shows a continuation of this tendency, the irregular cross septa here being more numerous and formed in the upper as well as the lower half of the spore, resulting in ten to fifteen transverse septa.

No. 57 represents a group of collections with red-brown spores showing extremely variable septation. The first three primary septa in these spores are usually thicker and more prominent. The secondary and tertiary cross walls laid down in the two central cells are thinner, often tardily formed, and irregular or incomplete, and extend between only two of several vertical septa. The two primary end cells in these spores tend to be irregularly septate by oblique septa. As a result, the septation in spores of a single mount may vary from seven to fifteen cross septa. Figure 47 is a spore of this type which might be referred to *P. montana* if it were not for the irregular septation. Figure 48 is a spore with a more complete septation, showing the three transverse septa in each of the central primary cells and the oblique septation of the end cells.



FIGS. 46-56. Spores of *Pleospora*.

The type collection of *P. alpestris* E. & E. is of this type, as is also a collection labeled *P. multiseptata,* on *Anthyllis,* which suggests that *P. Anthyllidis* Niessl is of this type.

The type collection of *P. amplispora* E. & E. (No. 65) has very large red-brown spores (FIG. 49) showing this type of three secondary and tertiary cross septa in one cell above the primary central septum and in two cells below this septum. The end cells are again obliquely septate in an irregular fashion. This results in spores with thirteen to eighteen or even more septa.

The type collection of *P. multiseptata* Starb. (No. 74) has very pale yellow-brown spores with fifteen or more transverse septa and are broken up into small cubical cells by numerous vertical walls.

No. 91 represents an undetermined collection on *Stipa,* which has somewhat flattened spores and which may belong in *Clathrospora.* In face view (FIG. 50) they have fifteen to sixteen transverse and five to six vertical walls which are very regularly formed. In edge view (FIG. 50a) the spores are narrower and show only scattered vertical walls which appear comparatively faint.

No. 59, which is shown as an offshoot of the herbaceous *herbarum* line, includes several western collections already referred to *P. obligasca* Bub. The spores (FIG. 56) are asymmetrically four- to six-septate, and although similar to this series in form, they are of a more simple type and do not seem to fit in with the rest of the series.

The *P. herbarum* types on woody stems are mostly at the seven-septate level and correspond to the *P. media* (Nos. 34 and 39) group. No. 51 refers to a group of collections with small, often clustered perithecia on shrubby hosts, and includes the type collections of *P. rubicola* Syd., *P. Ephedrae* Speg. and *P. Rhodotypi* Rehm (inedit.). The spores (FIG. 51) are broadly fusoid-ellipsoid and irregularly septate .

No. 47 refers to a group of collections on decorticated woody stems which were previously placed in the genus *Teichospora.* They were transfered to the genus *Pleospora* by Höhnel (**4**, p. 230) because the perithecia actually originate beneath the surface of the wood. The spores (FIG. 52) are almost identical with those of No. 51, although they tend to be somewhat narrower in comparison to their length. This group shows the close relationship between the

FIGS. 46–56. Spores of *Pleospora.*

woody forms of *Pleospora* and the genus *Teichospora*. The separation of the two genera will probably need revision after a study of these related forms.

No. 89 represents a group with very similar spores (FIG. 53) but much larger perithecia and found within the bark of woody stems. It includes the type collections of *P. laricina* Rehm and *P. pustulans* E. & E.

No. 11 is a collection of *P. Thuemeniana* Sacc., on *Agave*, which has a blackened clypeus about the large perithecia and has spores (FIG. 54) similar to the preceding, but shorter, stouter and with more rounded ends.

No. 80 is *P. Henningsiana* Ruhl, Jahn & Paul, which is a distinct species with large perithecia and seven-septate, elongate, cylindric spores with a gelatinous envelope (FIG. 55).

There are numerous collections with setose perithecia which have spores corresponding to the *herbarum* series. On herbaceous stems the perithecia are usually larger and more or less covered with a dense tomentum of stiff sinuous hairs. On the upper surface of such perithecia the hairs tend to become stiff, upright, pointed and to penetrate the overlying tissues. If soon freed from these overlying tissues these stiff hairs develop more abundantly, but they are easily broken off and it is a common experience to find only a few perithecia in a collection which show such setae. On leaves or minute stems there is another group in which the young perithecia are elongate-pyriform and have a rather prominent stout ostiolar neck from which there arise a cluster of pointed setae, whereas the base remains smooth. Such setose perithecia are usually found at high altitudes and are to be found under a multitude of binomials in herbaria. In Europe they are commonly referred to *P. helvetica* Niessl or *P. hispida* Niessl.

The spores of such collections show the same continuous series of overlapping variation which has been described for the *P. media–P. herbarum* series. By using a rather loose correlation of spore and perithecial characters an attempt has been made to separate certain specific (or varietal ?) groupings. Nos. 90, 53, 54 and 84 are a closely related group with five- or seven-septate spores with septate end cells, 19–26.5 × 7–10.5 μ, varying from yellow- to red-brown and from fusoid- to oblong-ellipsoid. In No. 90 the spores

are of the *vulgaris* type, similar to No. 81 but smaller and with vertically septate end cells. Nos. 53 and 54 both include collections with yellow-brown spores, which are more generally tapered in the former and with rounded ends in the latter. No. 84 includes forms with red-brown spores of variable form.

No. 56 represents a group of collections which show a similar spore-form but with spores slightly longer (21–28 × 9–12 μ), more oblong, and nearly always with seven septa. They are yellow- to red-brown.

No. 50 includes those collections with larger spores (26–37 × 11–16 μ) which correspond to *P. herbarum*. The spores of these collections are mostly dark yellow- to dark red-brown and often have the inequilateral form of the var. *occidentalis*, which is probably correlated with their occurrence at high altitudes.

No. 58 is the culmination of this series. In these collections the spores run still larger and show the irregular septation in the lower end with insertion of an eighth septum, as is found in No. 60.

No. 55 represents the type collection of *P. abbreviata* Fck., which shows spores with the irregular type of tertiary cross wall formation mentioned for No. 57. The perithecia are, however, of the conic, setose, leaf inhabiting type.

Many of the collections with setose perithecia are found on leaves. Most of the leaf inhabiting individuals, with spores of the *herbarum* series, are not distinct enough to be considered as separate groupings. No. 44, which is the type of *P. Inulae-candidae* Jaap, has the spores of the *P. media* (No. 39) type, but with the small perithecia imbedded in the dense mat of leaf hairs on the leaves of *Inula candida*. No. 45 consists of two collections, in Riksmuseet, labeled *P. phyllophyla* Rehm (inedit.) and has similar spores which, however, show very little or no constriction at the septa.

It is intended that the rather preliminary outline here presented will be supplemented in future papers by a more detailed presentation of the individual collections and species groups which will make possible the delimitation of species and application of binomials.

It can be seen, however, that there are certain tendencies which persist and are correlated with other varying factors which allow

the detection of related groups. The primitive type of septation which gave rise to the genus, for instance, was the occasional insertion of a vertical septum. This is found only in yellow-brown spores of rather narrow fusoid or oblong form. It persists during the formation of secondary septa and a five-septate spore, but as more septa are laid down (*i.e.*, in the asymmetrical leptosphaeroid branch) the *vulgaris* type of secondary septation is likely to occur.

This *vulgaris* type of secondary septation may occur in three-septate spores and is usually accompanied by a broader, inequilateral or curved spore form, which persists, without vertical septation of the end cells, even though nine to eleven transverse septa are formed.

Again, the *herbarum* series arises at the five-septate level by the further broadening of the spore and rounding of the ends. This rounding of the ends results in an increase in diameter of the end cells, which probably allows the formation of vertical septa in these cells, which in turn is characteristic of this series. As this type of spore increases in size, secondary and tertiary cross walls are formed until fifteen to eighteen transverse septa are found. The asymmetry of form of these spores with a longer lower end apparently arose first (in seven-septate spores) and was followed by the formation of additional septa in the lower portion, giving the asymmetrical septation characteristic of most of the members with numerous cross walls. This asymmetry as regards the formation of cross walls also occurs in both of the other series, but seems to be merely occasional rather than the dominant type of septation as it is in the larger spores of the *herbarum* series.

The clathrate spore forms (*Clathrospora*) are not discussed here, but it might be mentioned that they also show several distinct types of spore form, with a similar overlapping series of collections as regards spore size and septation for each.

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EXPLANATION OF FIGURES

FIG. 1. Basic spore form of the genus.

FIGS. 2–5. Fundamental modifications in spore form: 2, showing constrictions at septa; 3, inequilateral modification; 4, showing rounding of ends; 5, asymmetric development.

FIGS. 6–11. Generalized spore forms of the genus: 6, fusoid (leptosphaeroid type); 7, fusoid-ellipsoid (*vulgaris* type); 8, oblong-ellipsoid (*herbarum* type); 9, clavate, asymmetric type; 10, oblong type; 11, 11a, flattened clathrate (*Clathrospora* type).

*FIGS. 12–14. Leptosphaeroid (fusoid, symmetric) types: 12, spore of *Pleospora pellita* (Fr.) Rab.; 13, spore of undetermined collection (*P. vagans* Niessl), on *Phleum;* 14, spore from type collection of *Leptosphaeria Yerbae* Speg.

FIGS. 15–17. Leptosphaeroid (oblong) types: 15, spore from type collections of *P. mollis* Starb.; 16, spore from collection of *P. oblongata* Niessl, in the Rehm Herb.; 17, spore from another collection of *P. oblongata*, in Rehm Herb., labeled as a variety.

FIGS. 18–21. Leptosphaeroid (fusoid, asymmetric) types: 18, spore from type material of *P. Niessleana* Kze.; 18a, spore from collection of *P. dura* Niessl, on *Linaria;* 19, spore from collection of *P. dura*, on *Aconitum;* 20, spore from collection of *P. abscondita* Sacc. & Roum., on *Phragmites;* 21, spore of an undetermined collection on *Sorbus.*

FIGS. 22–23. Leptosphaeroid (fusoid-ellipsoid) types: 22, spore of an unpublished species, *P. Galii* Romell; 23, spore of an undetermined collection, on *Galium.*

FIGS. 24–34. Spores of the *vulgaris* series (symmetric types): 24, spore from type material of *P. oligostachyae* E. & E.; 25, spore of a collection of *P. diaporthoides*, on *Bardana;* 26, spore from type collection of *P. Boldoae* Speg.; 27, spore from type material of *P. infectoria* Fck.; 28, spore from type collection of *P. infectoria* var. *Sacchari* Speg.; 29, spore from a collection of *P. richtophensis* E. & E., on *Achillea;* 30, spore from a collection of *P. rubicunda* Niessl, on grass; 31, spore from collection of *P. Thurgoviana* Wegelin, on *Typha;* 32, spore from type collection of *P. Alismatis* E. & E.; 33, spore from collection of *P. Anthyllidis* var. *Aconiti* Rehm; 34, spore from type collection of *P. pleosphaeroides* Wehm.

FIGS. 35–38. Spores of the *vulgaris* series (asymmetric types): 35, spore from type collection of *P. kansensis* E. & E.; 36, spore from a collection of *P. herbarum* (Clem. Cr. Form, Colo. No. 442); 37, spore from a collection

* Figures 12–56 were drawn with a camera lucida, to the scale indicated on the plates.

of *P. Berberidis* Rab., in the Rehm Herb.; 38, spore from an undetermined collection on *Arenaria*.

FIGS. 39–45. Spores of *herbarum* series (on herbaceous stems): 39a, spore from a collection of *P. oblongata* Niessl; 39b, spore from a collection of *P. Maireana* Lamb. & Fautr., on *Laserpitium;* 39c, spore from a collection of *P. Compositarum* Earle, on *Penstemon;* 40a, spore from type collection of *P. Herniariae* Fck.; 40b, spore of *P. herbarum* (Fr.) Rab., from Rab., Herb. Myc., Ed. I, No. 547a; 40c, spore taken from the type of *P. herbarum* var. *occidentalis* Wehm.; 41, spore from type collection of *P. Jaapiana* Rehm; 42, spore from type collection of *P. maritima* Rehm; 43, spore from collection of *P. stenospora* Schroet., on *Rydbergia;* 44, spore from a collection of *P. njegusensis* Bub., on *Hedysarum;* 45, spore from type collection of *P. montana* Wehm.

FIGS. 46–49. Spores of the *herbarum* series (on herbaceous stems, with irregular septation): 46, spore from type collection of *P. longispora* Speg.; 47, spore from a collection of *P. montana* Wehm., on *Lupinus;* 48, spore from a collection of *P. multiseptata* Starb., on *Anthyllis;* 49, spore from the type collection of *P. amplispora* E. & E.

FIGS. 50, 50a. Face and edge views of a spore from an undetermined collection, on *Stipa*, showing flattened character of spores of the genus *Clathospora*, but with vertical septa in edge view.

FIGS. 51–55. Spores of the *herbarum* series (on woody stems): 51, spore from type collection of *P. rubicola* Syd.; 52, spore from a collection of *Pleospora obtusa* (Fck.) Höhn., f. *fibrincola* Höhn., on pasteboard; 53, spore from the type collection of *P. laricina* Rehm; 54, spore from a collection of *P. Thuemeniana* Sacc., on *Yucca* or *Agave;* 55, spore from a collection of *P. Henningsiana* Ruhl., Jahn & Paul, on *Salix*.

FIG. 56. Spore from Clem. Crypt. Form, Colo. No. 34 (*P. cybospora* Clem.), showing a simple type of asymmetric septation, with the *herbarum* form.

SOME LEAFSPOT FUNGI ON WESTERN GRAMINEAE—III

RODERICK SPRAGUE [1]

(WITH 2 FIGURES)

The fungi listed or discussed in this article were obtained in the far western United States during 1947. About eight hundred specimens were collected by G. W. Fischer, Jack Meiners, and the writer in Idaho, Utah, Arizona, New Mexico, California, Nevada, Oregon, and Washington during June 1947. In addition, the writer collected about 90 numbers in the Chelan National Forest north of Winthrop, Washington. Although the areas covered were extensive, the weather conditions, except in the Chelan region, were not favorable for leafspot development this year because of the shortage of seasonal rainfall. The following fungi are worthy of note:

Ascochyta utahensis sp. nov. Maculis brunneis, centro pallido; pycnidiis globosis, brunneis, pseudoparenchymatis, epiphyllis, ostiolatis, 113–148 μ; pycnosporulis elongato-ellipticis, cylindraceo-irregularibus, apicibus et basibus subobtusis, 1-septatis, contextu hyalino-chlorino, guttulato v. granuloso, 22.6–29 × 6.6–10 μ.

Hab. in foliis vivis et dejectis *Agropyri inermis* (Scribn. et Smith) Rydb., prope Logan, Utah. June 6, 1947. Sprague, Fischer, et Meiners coll. **Typus** est *C. S. 3610* [2] (Wash. State Coll. Dept. of Plant Pathology herbarium).

Spots chocolate brown, 1–6 × 1–3 mm., mostly elongate or forming irregular areas by fusion of several spots; pycnidia few, scattered, obscure, pseudoparenchymatous, brown, globose, ostiolate, 113–148 μ; pycnospores elongate-elliptical or cylindrical but tapering to blunt ends, straight or more often bent or slightly lumpy in outline, contents yellow to nearly hyaline, guttulate to granulose, one-septate (median septation), not or scarcely constricted at the septum, large, 22.6–29.2 × 6.6–10 μ.

[1] Associate pathologist, Div. of Plant Pathology, Washington State College. Published with the approval of the Director of the Washington Agric. Exp. Sta. as Technical Paper No. 745.

[2] C. S. refers to the Collection Series of the pathological collections of the Department of Plant Pathology, Washington State College, Pullman.

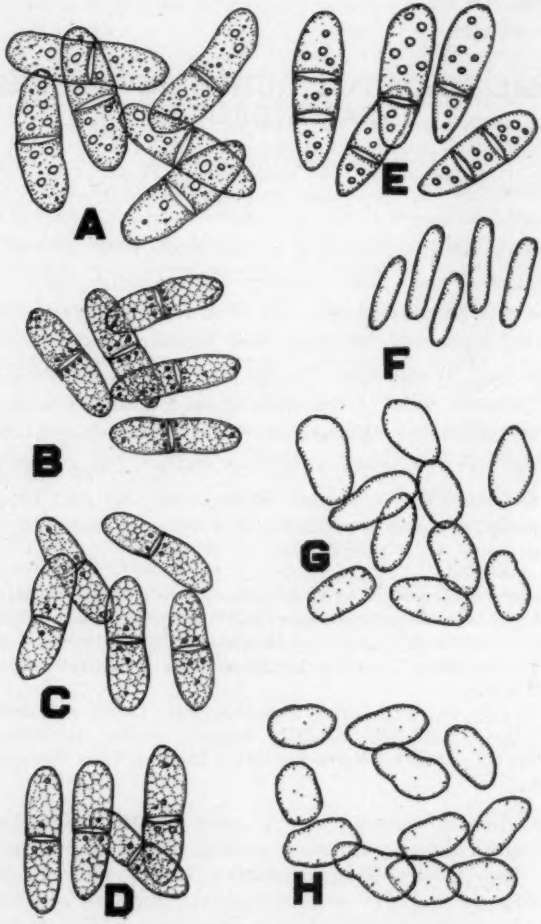

FIG. 1. Pycnospores of fungi on Gramineae.

This material was obtained on a steep, grassy slope in Logan Canyon near Logan, Utah, and consists of a number of leaves with well-defined spots which could be mistaken for those caused by *Pseudomonas coronafaciens* var. *atropurpureum* (R. and G.) Stapp. The pycnidia are scarcely discernible with a hand lens and are not

abundant. The spots which are brown when young eventually become pallid in the center.

This species has larger spores (FIG. 1, *a*) than any of the other species of *Ascochyta* on western grasses. In the writer's key to the species [3] it belongs in section *Ascochytula* and is probably closest morphologically to *Ascochytella avenae* Petrak.[4] However, even in winter material, the spores of the latter species measure only 17–26 × 6.2–7.0 μ, and, therefore, just exceed the minimum dimensions of those of *A. utahensis*. In addition, the symptoms of the latter appear very distinct from those of *A. avenae*.

A. utahensis was compared with *A. agropyrina* (Fairman) Trotter (**9**). This species, which occurs on *Elymus* and *Agropyron* in the Great Plains, has yellow spores measuring 14–19 × 4.5–6.2 μ in summer material. The spores of the latter are, therefore, much smaller and also tend to be more uniformly cylindrical. It is doubted if *A. utahensis* could be a giant phase of *A. agropyrina*. Therefore, it is described as distinct.

This fungus, collected during the Centennial commemorative year of the colonizing of Utah, is named for that state. One collection on *Agropyron smithii* Rydb. made near Belfield, North Dakota, June 19, 1945, is apparently the same species (*B. P. I. 81, 124*).

ASCOCHYTA HORDEI Hara. Hara has described (**12**) a species of *Ascochyta* on barley from Japan which is the same as a yellow-spored species from the western United States (FIGS. 1, *b*; 1, *c*; and 1, *d*).

Material on *Hordeum murinum* L. from near Riggins, Idaho (*C. S. 3677*), on *Bromus carinatus* Hook. and Arn. from Logan,

[3] Sprague, R. Rootrots and leafspots of grains and grasses in the northern Great Plains and western states. U.S.D.A. B.P.I.S.A.E. Plant Disease Reporter. Supplement 163. June 15, 1946. (Processed.)

[4] Ann. Myc. **23**: 107. 1925. This will probably be discussed in a later publication. *Ascochytella avenae* should be known as **Ascochyta avenae** (Petrak) Sprague and A. G. Johnson comb. nov. Spots usually vague necrotic areas, pycnidia prominent, dark brown, 100–140 μ (in western United States); pycnospores irregularly cylindrical to fusoid; ends rounded, spore contents coarsely granular, sometimes two-septate but usually with one median septation, 17–26 × 6.2–7 μ, tardily yellow. Spores sometimes smaller in summer material.

Utah (*C. S. 3852*), and on *Festuca elatior* L. near Logan, Utah (*C. S. 3656* and *3682*) carried what we call *Ascochyta hordei* with the following characters:

Spots few, whitish yellow to clay colored or vaguely pale, nearly white, later with brown margins or sometimes fading to vague necrotic areas; pycnidia thin-walled, parenchymatous, globose or slightly flattened, ostiolate, epiphyllous, erumpent, 100–172 μ; pycnospores chlorinous or very pale yellow to virtually hyaline, cylindrical with rounded ends or less often slightly twisted or semi-fusoid but scarcely fusoid and not tapering strongly towards either end, one- (seldom two)-septate, 16–22 × 4.8–6.2 μ on *Festuca* (FIG. 1, *b*) and *Hordeum* (FIG. 1, *c*); 17.8–21 × 5.3–7.1 μ on *Bromus* (FIG. 1, *d*).

APIOCARPELLA MACROSPORA (Speg.) Sydow (*Apiosporella macrospora* Speg.). Somewhat scanty material of this probably saprophytic species was collected on *Stiporyzopsis bloomeri* (Boland) Johnson [*Oryzopsis hymenoides* (Roem. and Schult.) Ricker × *Stipa occidentalis* Thurb.] at 8,000 feet on Mt. Whitney, California (*C. S. 3605*). The prominent, black, brittle pycnidia were in straw-colored areas on dead leaves. The pycnidia were at first immersed, but later, through erosion of the leaf epidermis, were superficial on the sub-epidermal layers. They were very flattened, about 80 μ tall, 160–350 μ long and 80–130 μ wide and had no ostiole until nearly mature. The spores were elliptical to elongate (narrowly obovate), with the basal (smaller cell) definitely pointed (FIG. 1, *e*). The eccentrically placed cross-wall is typical of the species. The spores were slightly greenish-hyaline with a number of medium-sized bodies in the cell contents, 21–26 × 6.6–8.8 μ. Spegazzini (**17**) described this species as *Apiosporella macrospora* in 1910, but, as the genus name had been used by v. Höhnel in 1909, Sydow (**23**) proposed *Apiocarpella*. Spegazzini described the species on *Hordeum jubatum* as having lenticular, prominent pycnidia, 150 μ in diameter, with coarse parenchymatous tissue, and ellipsoid-elongate conidia 28–30 × 7–8 μ, and with the lower cell of the spore much smaller than the upper. Without having the type available for critical comparison it seems conservative to consider our fungus the same as Spegazzini's, as the spores, except for being slightly shorter, have approximately the same dimensions.

H. C. Greene sent a specimen of *Apiocarpella* on *Calamagrostis canadensis* L. from Madison, Wisconsin, collected July 22, 1947. The fungus occurred in elongated, pale spots on living leaves. The spores were 13–16 × 3.7–5.0 μ and therefore were much smaller than those of *A. macrospora*. This fungus will be described as an autonomous species in one of Greene's papers on Wisconsin fungi.

Pyrenochaeta elymi sp. nov. Pycnidiis subgregariis, erumpentibus-superficialibus, globosis, ostiolatis, aureo-brunneis, v. brunneis prope ostiolis, contextu membranaceo-parenchymatico, 130–170 μ diam.; setulis 5–15, rigidis, septatis, acutis, socia ostiolis, 120–170 μ; pycnosporulis numerosis, continuis, hyalinis, cylindraceis, 13.2–15 × 2.6–3.4 μ.

Hab. in foliis dejectis, *Elymi glauci*, prope Logan, Utah.

Pycnidia several in gray, dead tissue, subgregarious, subsuperficial, globose, ostiolate, golden brown but with a contrasting dark brown ring around the slightly rostrate ostiole, membranaceous-parenchymatous walled, composed of squared cells as viewed in whole mount, 130–170 μ diameter but young pycnidia sometimes smaller; setae numerous, 5–15 in number, ringing ostiole and rising above pycnidium to a distance approximately equal to the height of the pycnidium, somewhat divergent, brown, septate, tapering to a point, rigid and spine-like, up to 170 μ long; pycnospores numerous, exuding in tendrils from the ostioles, hyaline or slightly smoky in mass, cylindrical but with rounded ends (bacillar) all nearly identical in size and shape, 13.2–15 × 2.6–3.4 μ (FIG. 1, *f*).

Hab. on dead leaf tips of *Elymus glaucus* in Logan Canyon, near Logan, Utah (*C. S. 3631*). Collected by Jack Meiners, G. W. Fischer and R. Sprague, June 6, 1947.

This interesting saprophyte appears to be distinct from any known species of this genus on grasses or sedges. *P. exosporioides* (Fckl.) Sacc. (**15**, v. 3, p. 221) has elliptical spores 10 × 3 μ. It occurs on *Carex*. The shape of the spores, if not their size, removes *P.* sp. from this species. *P. luzulae* (West.) Sacc. (*Vermicularia luzulae* West.) has globose spores 5 μ in diameter, and is even more distinct. *P. leptospora* Sacc. and Briard (**16**) has oblong to ovoid, cylindrical or obtuse spores, 4–5 × 1.5 μ, and occurs on *Milium effusum* L. *P. graminis* Ell. and Ev. (**8**) has pycnidia without ostioles and has short setae 20–40 × 3 μ, as well as globose or ovate spores 8–14 μ long. It occurs on dead foliage of *Chloris verticillata* Nutt. *P. oryzae* Miyake (**14**) is

closer to our material than most of the others in pycnidial characters, but the spores are fusoid, 4–6 × 1.5–2 μ.

Macrophoma sporoboli sp. nov. Pycnidiis non in maculis v. in maculis griseis, epiphyllis, erumpentibus, nigris, depresso-globosis v. ellipsoideis, carbonaceo-parenchymatis, ostiolatis (minute et tarde), 70–155 × 70–140 μ; pycnosporulis hyalinis v. opacis, aseptatis, variabilibus, ellipsoideis, v. subcylindraceis, obovatis, subfusoideis, 7.5–19 × 5.6–8.9 μ (13–17 × 6.6–7.8 μ).

Hab. in foliis emortuis *Sporoboli gigantei* Nash, prope Winslow, Arizona. Sprague, Fischer et Meiners, coll. June 11, 1947. **Typus** est *C. S. 3609;* et in *Sporobolo* sp. Hatch, New Mexico. Sprague, Fischer et Meiners, June 13, 1947 (*C. S. 3670*).

Pycnidia not in spots, or sometimes in gray lesions, epiphyllous, scattered, black, depressed-globose to less often somewhat ellipsoid, erumpent, carbonaceous-parenchymatous, 70–155 × 70–140 μ, ostiole minute or often very tardily formed; spores released in mounts by crushing the brittle pycnidia, pycnospores hyaline to somewhat opaque but not tinted (when examined six weeks after collecting), non-septate, variable in shape, generally elongate-ellipsoid or tapering towards one end and then obovate, sometimes ovate to nearly truncate, sometimes nearly cylindrical, a few small spores subglobose, others sub-fusoid, or even constricted towards one end and then roughly broadly pyriform, 7.5–19 × 5.6–8.9 μ but typically 13–17 × 6.6–7.8 μ.

On dead leaves of *Sporobolus giganteus* Nash, 5 miles east of Winslow, Arizona (*C. S. 3609,* **Type**), and on *Sporobolus* sp. Hatch, New Mexico (*C. S. 3670*).

The type appears to be one of several saprophytes on the leaves of the conspicuous giant drop-seed growing along the main highway east of Winslow. A species of *Leptosphaeria* is associated with the fungus.

At the time examined, this fungus had hyaline spores (FIGS. 1, *g*; 1, *h*) with the typically opaque, non-guttulate contents of the spores of fungi which we have placed in *Macrophoma.* There was no indication of color (*Sphaeropsis*) in the spores at the time examined. However, it might be open to question as to why this should not be placed in *Phyllosticta.* The spores, we believe, are somewhat large for *Phyllosticta,* and, in addition, the absence or vagueness of spots is possibly more like *Macrophoma.* Furthermore, the opaque (or "frosty"), non-guttulate aspect of the spore contents is nearer

to that of *M. phlei* Tehon and Stout, for instance, than to that of *P. rogleri* Sprague, a large-spored *Phyllosticta*. *M. sporoboli* differs from *M. phlei* in having distinctly smaller polymorphic spores. Although many of the spores are typically elongate to egg-shaped, others vary from pyriform to nearly globose.

The material on *Sporobolus* sp. from Hatch, New Mexico, had gray, vague, ellipsoidal spots, 2–4 × 0.5–1.5 mm. The pycnidia were black, subimmersed in lines, globose, non-ostiolate (? immature, scarcely *Sclerophoma*), 120–145 μ in diameter, and the spores had the same multiplicity of shapes as found in the type of *M. sporoboli*. In the Hatch material they measured 11–16 × 6.6–8.9 μ (FIG. 1, *g*). Therefore, the two collections are apparently co-specific, the only real difference being in the absence of a definite ostiole in the Hatch material. Ostioles are also sometimes absent or vague in the Winslow material, appearing to be scarcely more than a localized decadence of the pycnidial dome (compare FIGS. 1, *g*, and 1, *h*).

SEPTORIA AVENAE Frank on FESTUCA ELATIOR L. *Festuca elatior* is seldom attacked by leaf-spotting fungi. Therefore, the presence of a dark purple spot on leaves of *Festuca elatior* L. from Logan Canyon, Utah, is of interest. The causal organism appears to be an *Ascochyta* stage of *S. avenae*. The spores are mostly one-septate, rarely two- to three-septate, cylindrical, hyaline, 16.8–27.2 × 3.1–4.7 μ (FIG. 2, *a*). They are too large for any phase of *Ascochyta graminicola* Sacc., and are perhaps not large enough for *Stagonospora arenaria* Sacc. (18). Such specimens as this one are too similar to summer material of *Septoria nodorum* Berk., *S. avenae*, *S. secalis* var. *stipae* Sprague and others to justify describing them as new. All of these, however, except *Stagonospora arenaria* and *Septoria avenae*, must be eliminated from consideration because of the width of the spores of the fescue material.

This material on *F. elatior* was found on a lush prairie slope above Logan Creek in Logan Canyon, Utah, where the host was associated with *Agropyron inerme* (cfr. *Ascochyta utahensis*), *Melica* sp., *Festuca kingii* (S. Wats.) Cassidy (cfr. *Ovularia hordei*), *Balsamorhiza* sp., *Symphoricarpos oreophilus* A. Gray, *Castilleja* spp., and other mountain plants.

Material of *S. avenae* Frank on *Glyceria pauciflora* Presl. was found in a boggy, wooded area on Andrew's Creek, Chelan National Forest, Washington, August 9, 1947 (*C. S. 3871*). The spots were gray, elliptical to striate, later fading to straw-color, and with wide surrounding yellow areas. A second collection from the same area (*C. S. 3879*) showed lesions which were similar, except that mature ones were nearly buff in color. The pycnidia were yellow-brown under a 16 mm. lens, very thin-walled, flattened-globose, up to 150 μ in diameter, and ostiolate. Some of them were almost acervulus-like. This refers one to *Cylindrosporium glyceriae* Ell. and Ev. which has spores 15–30 × 2.5–3 μ. The status of this little known species is uncertain, however. Our material has true pycnidia, and the spores are cylindrical, three-septate, and 35–46 × 3.4–4.1 μ. The fungus appears to be close to, if not identical with, *S. avenae* Frank. The shaded, moist environment may have contributed to the fragile type of pycnidia. The fungus is distinct from our *S. glycericola* (**22**), which has smaller spores (20–34 × 1.9–3.4 μ), with a distinctive, sub-acute apex. Spores of *S. avenae* on this host are truly cylindrical. *S. glycericola* produces suborbicular buff spots.

There is a char spot (? *Euryachora* sp.) with immature, black, globose fruiting bodies associated with *S. avenae* on this *Glyceria* material (*C. S. 3872*).

A collection on *Hordeum nodosum* L., made five miles south of Donnelly, Idaho (*C. S. 3629*), has bacillar one-septate spores, 15–23.6 × 2.4–3.0 μ, borne in brown, elliptical spots on living leaves. This appears to be *S. nodorum* Berk.

SEPTORIA ELYMI Ell. and Ev. Additional material of a *Septoria* on *Agropyron smithii* Rydb., from the vicinity of Logan, Utah (*C. S. 3648*), is assigned to the large, curved to sinuous form of *S. elymi* (FIG. 2, *b*). *S. elymi* proper has comparatively straight, stiff spores. However, when spores of this type elongate in a pycnidium they become curved or sinuous and often lose most of their resemblance to the smaller, straighter type. This appears to be the case with *S. elymi* as it occurs on *A. smithii* (North Dakota, Utah), *E. canadensis* var. *robustus* (Scribn. and Sm.) Macken. and Bush (Iowa), and *E. glaucus* Buckl. (Michigan). Davis (**5**) ten-

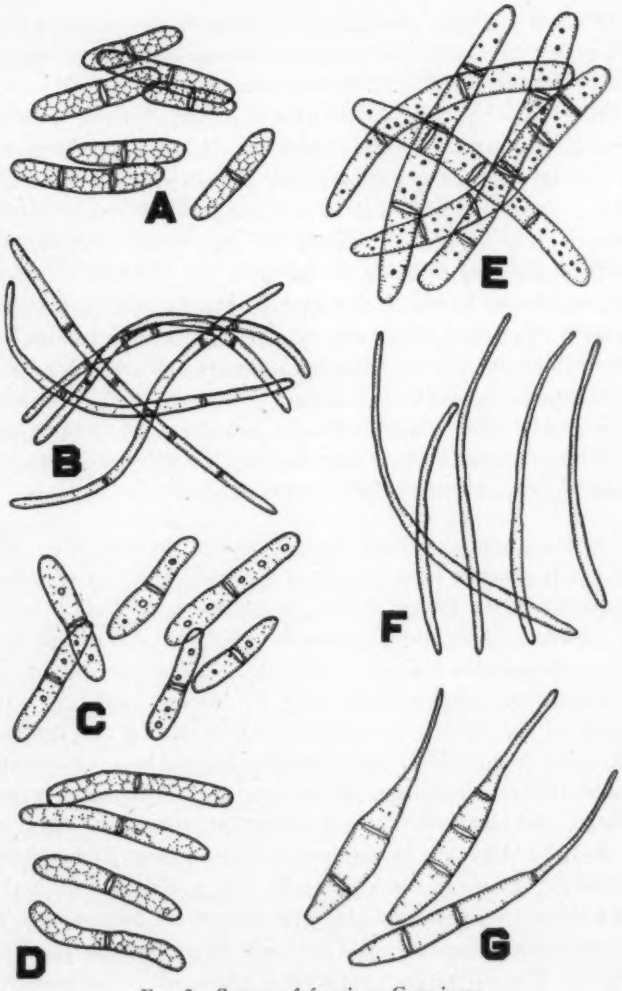

FIG. 2. Spores of fungi on Gramineae.

tatively described this form as *S. bromi* var. *clymina* on *E. virginicus* L. from Wisconsin. He retained this name in his final publication (**7**). Since this fungus tends to retain its large spore size in pure culture, it is possibly worthy of recognition. The writer

questions if this phase necessitates a formal name. But for those who prefer names for forms of species of fungi he proposes *Septoria elymi* forma **elymina** (J. J. Davis) comb. nov.

This phase of *S. elymi* differs from *S. pacifica* Sprague in having curved, not straight or stiffly curved, spores, in the type of growth on nutrient agar, and in the paler, very distinct pycnidia. It differs from *S. infuscans* (Ell. and Ev.) Sprague for the same general reasons. The complex on *Elymus* and *Agropyron* is difficult even for those critically studying the group.

S. agropyrina Lobik, *S. nodorum, S. avenae,* and *Stagonospora arenaria* all produce white cottony mycelium in pure culture, and their spores are coarser, ranging from coarsely obclavate in *S. agropyrina* to bacillar in *S. nodorum.* All occur, or have been reported, on *Elymus* and *Agropyron.* Frandsen (**10**) has recently described *S. elymina* (for *S. elymi* Rostr.), which has short, club-shaped spores and is very distinct from *S. elymi.*

SEPTORIA NODORUM Berk. on FESTUCA SUBULATA Trin. This is a locally common brown blight or leaf scald which covers a high percentage of the foliage of this grass growing in deep fir woods on Andrew's Creek in the Chelan National Forest north of Winthrop, Washington (*C. S. 3789, 3880*). Pycnidia are golden brown, globose, erumpent, 90–130 μ in diameter, and with small ostioles. The spores are hyaline, sub-cylindrical to fusiform-cylindrical, and straight, with somewhat blunted bases and tapered blunted apices. They are one-septate, slightly to scarcely constricted, and the contents are somewhat guttulate. They are 21–30 $\times$ 3.1–4.8 μ, and hyaline but with faint tinting of the contents. The writer assigns this to *S. nodorum* with considerable hesitation, not because the spores are only one-septate, but because they are proportionately too broad and fusiform for typical spores of this species. The difference is apparent if spores of *S. nodorum* from *Festuca subulata* (FIG. 2, *c*) are compared with more typical ones of *S. nodorum* from *Elymus glaucus* Buckl. (also a new host; *C. S. 3780,* FIG. 2, *d*). However, *S. nodorum* is somewhat variable, especially on *Triticum aestivum* L., so it appears conservative to assign the material on fescue to that species. The spores of

S. nodorum on *F. subulata* are the same size as those of *S. avenae* on *F. elatior* (FIG. 2, *a*) but less cylindrical.

In addition, W. B. Cooke collected necrotic material of *Melica californica* Scribn. which had scattered pycnidia of *S. nodorum* on the leaves. The spores were *Ascochyta*-like, sub-cylindrical, one-septate, and measure 17–25 × 2.8–3.5 μ. Of course this fungus could be an *Ascochyta* phase of *Septoria melicae* Pass. (*S. avenae*), but, in the absence of any evidence that *S. melicae* occurs in the region, it is, perhaps, more conservative to class it with *S. nodorum*.

SEPTORIA CALAMAGROSTIDIS (Lib.) Sacc. on TRISETUM SPICATUM (L.) Richt. Material of this species on the leaves of *Trisetum spicatum* from Andrew's Creek, Chelan National Forest, Washington (*C. S. 3879*) should be mentioned because the fungus shows some distinct differences from material on *Trisetum* spp. from Oregon (**20**). The spots are white to nearly straw color or sometimes faintly flesh color and have a narrow reddish border. Oregon material showed grayer spots with less distinct borders. The pycnidia in the Chelan material are large, as much as 210 μ in diameter, globose to ellipsoid, erumpent, and ostiolate. They are thin-walled, and many of them are eroded or eaten away with only remnants of the bases left. The spores are long, 62–92 μ, and wider than any material seen on *Agrostis* or *Calamagrostis*, the narrowest of them measuring 1.7 μ wide, about equal to the widest spores seen on *Agrostis* from Oregon. The spores are filiform, with narrowed apices, and slightly swollen, but tapering bases. They range to 2.4 μ in diameter. It appears conservative to place this collection in *S. calamagrostidis*. This is the second collection of a filiform-spored *Septoria* on *Trisetum spicatum* made in the state. The other collection, which was obtained on Mt. Rainier, has narrower spores. Possibly it should be compared with *Cylindrosporium calamagrostidis* Ell. and Ev. because the spore sizes are similar.

STAGONOSPORA ARENARIA Sacc. on DACTYLIS GLOMERATA L. In an earlier paper (**18**) the writer reported this species on *Dactylis* from Ohio and Oregon with one- to three-septate spores, 29–46 × 3.8–4.6 μ. Recent material was collected by George Nyland, Seth Barton Locke, and Avery Rich near Crescent Lake, Washington,

which showed vinaceous, indeterminate lesions bearing globose, scattered, golden-brown pycnidia, 95–160 μ in diameter. The spores were typical of those illustrated earlier but were somewhat larger, 40–52 × 3.5–5.3 μ, and sometimes five-septate (FIG. 2, *e*). There is some possibility of this fungus finally developing seven-septate spores, which would remove it from *S. arenaria*. This collection, however, must be assigned to *S. arenaria* on the basis of known morphology. In the light of revisions which we have made since our earlier paper appeared, it is considered desirable to mention this specimen and to point out that it can still be assigned to *S. arenaria*. Of the material originally assigned to *S. arenaria*, collections on *Arrhenatherum* (**18**) have been placed in *Septoria avenae* Frank, and some collections with pointed apices on *Elymus glaucus* (**18**, FIG. 2, *a*) were assigned to *Septoria agropyrina* Lobik. Collections on *Phalaris arundinacea* L. were found to be immature material of *Stagonospora foliicola* (Bres.) Bubak (**20**), although in the original condition seen they were excellent facsimiles of *St. arenaria* (compare **17**, FIG. 2, *e*, *f* and **20**, FIG. 2, *a*).

After excluding the specimens on *Phalaris*, *Scolochloa*, *Arrhenatherum* and a portion of those on *Elymus glaucus*, we have *S. arenaria* listed on the following hosts by states: *Agropyron ciliare* (Trin.) Franch, N. Dak.; *A. cristatum* (L.) Gaertn., N. Dak.; *A. desertorum* (Fisch.) Schult., N. Dak.; *A. repens* (L.) Beauv., Ore.; *A. smithii* Rydb., N. Dak.; *A. spicatum* (Pursh) Scribn. and Sm., N. Dak.; *A. trachycaulum* (Lk.) Malte, N. Dak.; *A. trichophorum* (Lk.) Richt., N. Dak.; *Cinna latifolia* (Trev.) Griseb., Ore., Minn.; *Dactylis glomerata* L., Ore., Wash., Ohio; *Elymus antarcticus* Hook. f., Wash.; *E. canadensis* L., N. Dak.; *E. dahuricus* Turcz., N. Dak.; *E. glaucus* Buckl., Ore., Wash.; *E. junceus* Fisch., N. Dak.; *E. mollis* Trin., Alaska; *E. pseudoagropyron* (Griseb.) Trin., N. Dak.; *E. sibiricus* L., N. Dak.; *E. triticoides* Buckl., Calif.; *Oryzopsis hymenoides* (Roem. and Schult.) Ricker, Mont. A collection on *Puccinellia nuttalliana* (Schult.) Hitchc., from Crystal Springs, N. Dak., is excluded and probably is closer to *Septoria*. It may, perhaps, be undescribed.

PHAEOSEPTORIA FESTUCAE Sprague. Material collected on *Festuca ovina* var. *brachyphylla* (Schult.) Piper at Tioga Pass, Cali-

fornia (*C. S. 3561*), is referable to *P. festucae*. It should be mentioned because it varies from the type on *F. rubra* L. (**19**). The spores are light yellow, filiform, and very long, 75–115 × 2.4–3.4 μ and seven- to eleven-septate. The measurements of the spores in this collection, therefore, mostly range larger than those in the type. The pycnidia are brown, strongly erumpent, more or less gregarious, and 100–160 μ in diameter. The pycnidia, therefore, mostly range in size above the upper limits of the measurements given for this species (**19**).

Since *P. festucae* and its varieties indicate or possess variable morphology, it is believed logical to include this alpine form in the species. It is associated with *Pyrenophora* sp., *Selenophoma everhartii* (Sacc. and Sydow) Sprague and A. G. Johnson, *Lophodermium arundinaceum* (Schrad.) Chev., *Cladosporium herbarum* Lk. (*Mycosphaerella tulasnei* Jancz.) and traces of *Hendersonia simplex* Schrtr. All of these occur on the dead, overwintered leaves of plants in early head collected June 22, 1947. The plants were growing at an elevation of 10,000 feet at the eastern entrance to Yosemite National Park.

Similar material was found on dead leaves of *Calamagrostis rubescens* Buckl. collected at 5,500 feet elevation above Andrew's Creek, Chelan National Forest, Washington (*C. S. 3873*). The spores had the typical shape of those of *P. festucae* rather than of the vermisporous ones of *P. calamagrostidis* which occurs on *Calamagrostis* on the seacoast. The spores of the Chelan forest material were 60–98 × 3.4–4.6 μ, light yellow-brown, and seven- to eleven-septate.

CYLINDROSPORIUM CALAMAGROSTIDIS Ell. and Ev. Material of this species on *Muhlenbergia filiformis* (Thurb.) Rydb. was found along Diamond Creek at Palmyra forest camp in the Uinta National Forest, Utah, June 7, 1947 (*C. S. 3685*). The very small leaves which bore the fruiting bodies of the fungus were yellow, but the lesions themselves were vague brown areas with faintly browner margins. The olive-brown, seriately arranged acervuli were 80–170 × 50–80 μ. They were erumpent and consisted of truncated, open bodies which scarcely resembled pycnidia as the entire distal third or half of each pycnidium was missing. Dried

spore masses of a resinous, amber color covered the bodies and extended for a short distance beyond their margins. The spores were hyaline, filiform, whip-like (flagelliform), with the basal half very narrowly obclavulate and the apex curving into a very fine tip. The spores were guttulate and questionably one- to three-septate or falsely divided, 50–72 × 1.7–2.5 μ (FIG. 2, *f*).

C. calamagrostidis was described as having filiform spores, 40–60 × 1.5–2.0 μ, borne in acervuli, 150–200 μ. The material on *Muhlenbergia* appears to be morphologically the same as the type on *Calamagrostis*.

SPERMOSPORA SUBULATA (Sprague) Sprague on CALAMAGROSTIS. Fragmentary material on pinegrass (*Calamagrostis rubescens* Buckl.) was collected on Andrew's Creek, Chelan National Forest, Washington (*C. S. 3869*), August 11, 1947. The three-septate spores are typical of *S. subulata*, except for size. They measure 50–80 × 7.0–8.4 μ. The type on *Melica* has spores only 20–35 × 2.5–4.3 μ, but on red fescue and on *Deschampsia* the spores range up to 50 × 4.6 μ. *Calamagrostis* represents an entirely new tribe (*Agrostidae*) for this genus. It has been reported previously on tribes *Festucae* (*Melica* and *Festuca*) and *Avenae* (*Deschampsia*). Since the material on pinegrass consists of only two leaves, it is not possible to study adequately this form with the exceptionally large spores. Because it appears to differ only in relative size (twice as long and twice as wide), it is placed in *S. subulata* (FIG. 2, *g*). It was associated with undeveloped fruiting bodies of, perhaps, *Mycosphaerella tulasnei*.

It might be mentioned in passing that a spore of a *Spermospora*-like fungus was noted associated with a char spot of *Glyceria pauciflora* Presl. (*C. S. 3872*). This spore was clear, hyaline, about 80 μ long and vaguely resembled a very narrow spore of *Mastigosporium* with a lone apical appendage. This may have been a stray parasite on the char spot. Davis (3) reported *Piricularia parasitica* Ell. and Ev. with spores up to 50 μ long and with the apex tapering into a narrow portion, but examination of Davis' collection, sent by H. C. Greene, shows an entirely different fungus.

OVULARIA PUSILLA (Ung.) Sacc. and D. Sacc. [*Ramularia pusilla* Ung., ?*Caeoma pusillum* Bonord., *R. pulchella* Cesati, *O. pulchella* (Ces.) Sacc.].

This fungus is generally known as *O. pulchella,* but it appears that the correct name is *O. pusilla.* The difficulty relative to *O. pusilla* stems partly from an indexing error by Saccardo, who confused *Ovularia haplospora* (Speg.) P. Magn. on *Alchemilla vulgaris* L. with *Ramularia pusilla* Ung. described in 1833 on *Poa nemoralis* L. (**24**). Lindau (**13**) pointed this out, and the error is acknowledged by Saccardo (**15**, v. 18, p. 532). *O. pusilla* is listed by Saccardo and D. Saccardo (**15**, v. 18, p. 531) as having erect, subramose, caespitose hyphae, arising from stomata and acrogenous, ovate-ellipsoid, non-septate spores. The spore dimensions are lacking but Saccardo states that on checking with figure 10 of Unger's Table II, the dimensions are approximately 8–10 × 5–6 μ. All evidence indicates that *O. pusilla* is an earlier name than *O. pulchella,* in which case, the latter should be reduced to synonymy.

O. pusilla was collected in Lawyer Canyon State Park, Idaho, June 4, 1947 (*C. S. 3641*), on an unreported host, *Bromus inermis* Leyss. The spots were small, 1–2 mm. in diameter, mostly circular or sometimes elliptical, gray-buff with a narrow brown border sometimes as broad as the small lighter center. Sometimes ochre or yellow necrotic areas formed around the lesions and were numerous enough to impair photosynthetic activity of the plant. The conidiophores were very obscure and often absent, but in the larger spots they were visible under a low-power binocular as fragile, hyaline structures. A few ovate, crystal-hyaline spores were produced which were 8.5–10.7 × 5.7–6.8 μ when mature. The fungus is readily referable to *O. pusilla,* and it is mentioned here because of its presence on this important forage grass. Several years ago we found *O. pusilla* on *B. carinatus* near Celilo Falls in Washington. The spots were small and gray. A suitable common name for this disease on *Bromus* spp. would be "round eye spot."

OVULARIA HORDEI (Cav.) Sprague. Cavara (**1**) reported spores of *Ovularia hordei* measuring only 6–8 × 4.5 μ on *Hordeum*

but with characteristic, serpentinous conidiophores and spores with evident walls. Davis (**6**), Greene (**11**), and Sprague (**21**) report similar material on *Phalaris arundinacea*, Sprague, however, giving larger spore measurements (11–15.5 × 6–7 μ). Also, *O. baldingerae* Eliasson has been described on *Phalaris* with spores 9–12 × 6–7 μ, intermediate in length between Cavara's measurements and Sprague's. These measurements are very close to *O. pulchella* var. *agropyri* Davis, 9–12 × 6–9 μ (**4**). Davis apparently later discarded this variety (**7**). It appears difficult to segregate *O. hordei* from *O. pusilla* (*pulchella*) on spore size. The tortuous aspect of the conidiophores of *O. hordei* plus the evident spore wall are of diagnostic value. However, material recently collected on *Festuca kingii* in Logan Canyon, Utah (*C. S. 3851*) shows conidiophores 30–40 × 3.5 μ with less serpentine traits, some being nearly straight, some moderately twisted. The spores are 16–22 × 9.5–10.5 μ, *i.e.*, larger than *O. hordei* on *Phalaris*. In addition, the faint tendency to muriculations seen in the *Phalaris* material becomes more pronounced in the *Festuca* collections. This brings us to *Ovularia holci-lanati* Cav. (**2**), which Lindau places in the spiny-spored genus *Ramulaspera* (**13**) as *R. holci-lanati*. The spores of this species are 16–27 × 6–10 μ but the conidiophores are given as 170 × 2 μ. These seem abnormally long, but, in the absence of type material, our fescue material cannot logically be assigned to *R. holci-lanati*. In addition, the writer questions if the spores of the fescue collection are spiny enough to be relegated to *Ramulaspera*. It should be added, however, that scattered, yellowish, definitely spiny spores do occur with the minutely spiny, hyaline ones, and only careful culture study can satisfactorily determine this point. It is not impossible that *O. pusilla, O. hordei, O. lolii* Volk, and *R. holci-lanati* are phases of the same *Ramularia*-like species. At present, it is necessary to recognize all except *O. lolii* Volk, which apparently is the same as the material on *Phalaris* and is assigned to our somewhat emended concept of *O. hordei*. The material on spike fescue is left in *O. hordei*. An English description is appended, but no formal name is given:

Spots burnt umber with a tardily paler, nearly white center, spots elongate-elliptical to linear, 2–4 × 0.5–1 mm.; conidiophores few, somewhat arranged in lines, mostly hypophyllous, fascicled and arising from a clump of hyaline cells averaging 14 × 14 μ, several conidiophores from each clump, 30–40 × 3.5 μ, tapering to a sharp apex or somewhat serpentinous in some older stalks, spores ellipsoid-ovate, definitely elongated, usually much narrower at one end, 16–22 × 9.5–10.5 μ, hyaline, with a distinct wall which is minutely echinulate.

LOPHODERMIUM ARUNDINACEUM (Schrad.) Chev. Scattered, large, elliptical perithecia occur as relatively prominent, elongate, black bodies on the white, bleached, overwintered leaves and stems of many alpine and subalpine species of grasses in Utah, Arizona, Washington, and California. The fungus at Tioga Pass, California, at 10,000 feet was sometimes associated with a snow mold, but all evidence indicates that *L. arundinaceum* is a saprophyte. The perithecia ranged up to 700 × 300 μ. The elongated, slit-like ostiole is characteristic. Although the fungus is almost omnipresent on grasses at Tioga Pass, collections were not made on all of them. The following collections or notations were made there and elsewhere.

Agropyron inerme (Scribn. and Sm.) Rydb., Logan Canyon, Utah (*C. S. 3657*); *Agrostis oregonensis* Vasey, Andrew's Creek, Wash. (*C. S. 3873*), on old stems mixed with new growth parasitized by *Septogloeum oxysporum* Sacc., Bomm. and Rouss.; *Calamagrostis breweri* Thurb., Tioga Pass (*C. S. 3491, 3567*); *Danthonia intermedia* Vasey, Tioga Pass Road (*C. S. 3689*); *Festuca arizonica* Vasey, Kaibab National Forest, Ariz.; *F. elatior* L., Tioga Pass; *Festuca ovina* L. var. *brachyphylla* (Schult) Piper, Tioga Pass (*C. S. 3751*); *Muhlenbergia* sp., Mt. Whitney, Calif., at 9,000 and at 9,300 feet (*C. S. 3506*); *Oryzopsis exigua* Thurb., Chelan National Forest, on Andrew's Creek (*C. S. 3698*); *Poa compressa* L., Tioga Pass Road; *P. epilis* Scribn., Tioga Pass (*C. S. 3574, C. S. 3818*); *P. fendleriana* (Steud.) Vasey, Jacob's Lake, Ariz. (*C. S. 3550*); Bryce National Park, Utah (*C. S. 3674*); *P. juncifolia* Scribn., Kaibab National Forest, Ariz. (*C. S. 3532*); *P. leibergii* Scribn., Tioga Pass (*C. S. 3568, 3581*), Tioga public camp, Calif. (*C. S. 3866*); *P. longiligula* Scribn. and Wils., Grand Canyon Park, north rim, Ariz. (*C. S. 3549*); *P. pratensis* L., Tioga Pass Road, McCall, Idaho; *P. secunda* Presl., Bryce National Park (*C. S. 3527*), Grand Canyon Park, north rim, Ariz. (*C. S. 3545*); *P. rupicola* Nash, Tioga Pass (*C. S. 3572*); *Sitanion hystrix* (Nutt.) J. G. Sm., Tioga Pass Road at 8,900 feet (trace only) (*C. S. 3578*); Grand Canyon, Ariz. (*C. S. 3595*); *Trisetum spicatum* (L.) Richt., Tioga Pass (*C. S. 3579*).

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EXPLANATION OF FIGURES

All illustrations made with the aid of the camera lucida. All drawings × 1,000.

FIG. 1. Pycnospores of fungi on Gramineae. *a*, From type of *Ascochyta utahensis* on *Agropyron inerme*, Logan Canyon, Utah (*C. S. 3610*). *b*, *A. hordei* Hara on *Festuca elatior*, Logan Canyon, Utah (*C. S. 3656*). *c*, *A. hordei* on *Hordeum murinum*, Riggins, Idaho (*C. S. 3677*). *d*, *A. hordei* on *Bromus carinatus*, Logan, Utah (*C. S. 3852*). *e*, *Apiocarpella macrospora* on *Stiporyzopsis bloomeri*, Mt. Whitney, Calif. (*C. S. 3605*). *f*. Pycnospores of *Pyrenochaeta* sp., type (*C. S. 3631*). *g*, Pycnospores of *Macrophoma sporoboli* on *Sporobolus* sp., Hatch, New Mexico (*C. S. 3760*). *h*, Pycnospores of *Macrophoma sporoboli* on *Sporobolus giganteus* near Winslow, Ariz., type (*C. S. 3609*).

FIG. 2. Spores of fungi on Gramineae. *a*, *Septoria avenae* on *Festuca elatior*, Logan Canyon, Utah (*C. S. 3624*). *b*, *Septoria elymi* on *Agropyron smithii* north of Logan, Utah (*C. S. 3468*). *c*, Pycnospores of *Septoria nodorum* on *Festuca subulata* (*C. S. 3789*). *d*, Pycnospores of *S. nodorum* on *Elymus glaucus*, Lake Quinault, Wash. (*C. S. 3780*). *e*, Pycnospores of *Stagonospora arenaria* on *Dactylis glomerata*, near Crescent Lake, Wash. *f*, Conidia of *Cylindrosporium calamagrostidis* on *Muhlenbergia filiformis*, Uinta National Forest, Utah (*C. S. 3685*). *g*, Conidia of *Spermaspora subulata* on *Calamagrostis rubescens*, Andrew's Creek, Chelan National Forest, Wash. (*C. S. 3869*).

NOTES ON THE PARASITIC FUNGI OF ILLINOIS [1]

LEO R. TEHON

(WITH 15 FIGURES)

In this paper, as in earlier numbers of the series, are described and named as new species a number of plant-inhabiting, presumably parasitic, fungi collected in Illinois. Included also are notes on host range and distribution and critical comments on the differentiation of old species related to those herein described.

Host nomenclature, in the main that of Gray's New Manual of Botany, Seventh Edition, and Bailey's Manual of Cultivated Plants, for wild and cultivated plants respectively, departs in some instances, *e.g., Iris Shrevei* Small, in favor of accepted names.

Cited specimens are designated by places and dates of collection and their accession numbers in the Mycological Collection of the Illinois State Natural History Survey, in which they are deposited together with the original or representative microscopical slides upon which these studies were developed.

Leptothyrium anthelmintici sp. nov.

Maculis in caulibus hospitis effusis, non limitatis; pycnidiis dimidiatis, rotundatis, bene fornicatis, nigris, nitidis, subcuticularibus, contextu pseudoparenchymato non radiante, saepe fimbriatis hyphis brunneis subcuticularibus, 125–150 μ diametris, poro centrali rotundo 10–15 μ lato apertis; sporis hyalinis, continuis, oblongis vel pediformibus, superne rotundatis, basim leniter attenuatis, 18–23 $\times$ 5–7 μ.

Caulicolous, in extensive, unlimited pale tan areas; pycnidia dimidiate, round, well arched, black, shining, subcuticular, pseudoparenchymatous, not radiate, often fimbriate with few to many brown, long, subcuticular hyphae, 125–150 μ in diameter, opening by a central, round pore 10–15 μ wide; spores hyaline, nonseptate, oblong or pediform, the distal end bluntly rounded, the basal end somewhat tapered, 18–23 $\times$ 5–7 μ.

[1] Earlier *Notes* in this series appear in Mycologia as follows: **16**: 135–142. 1924; **17**: 240–249. 1925; **19**: 110–129. 1927; **21**: 180–196. 1929; **25**: 237–257. 1933; **29**: 434–446. 1937.

On dead stems of *Chenopodium ambrosioides anthelminticum* (L.) Gray, Harrisburg, Saline County, Ill., August 16, 1943, Acc. No. *30049* (**type**).

Although also a stem inhabitant, this second species of *Leptothyrium* to be recorded on *Chenopodium* differs from *L. Chenopodii* Dearn. in that its pycnidia are somewhat smaller while its spores, differently shaped, are three to five times as long.

Leptothyrium Avenae sp. nov.

Pycnidiis sub stomatibus in glumis et pedi hospitis seriatis sitis, subcuticularibus, nigris, immersis, cum cellis externis aliformibus et radiatis, plerumque late ellipticis, 100–200 × 65–140 μ; poro minuto sub stomati apertis; sporis hyalinis, continuis, ovalibus, oblongis vel pediformibus, 4–8.5 × 16–23 μ; sporophoris simplicibus, brevibus, acutis.

Pycnidia substomatal, in rows on the outer faces of glumes and on pedicels, subcuticular, black, remaining immersed, with cells of the outer wall aliform and radiate, for the most part broadly elliptical, sometimes nearly round, 100–200 × 65–140 μ; pore minute, substomatal; spores hyaline, nonseptate, oval, oblong or pediform, 4–8.5 × 16–23 μ; sporophores simple, short, acute.

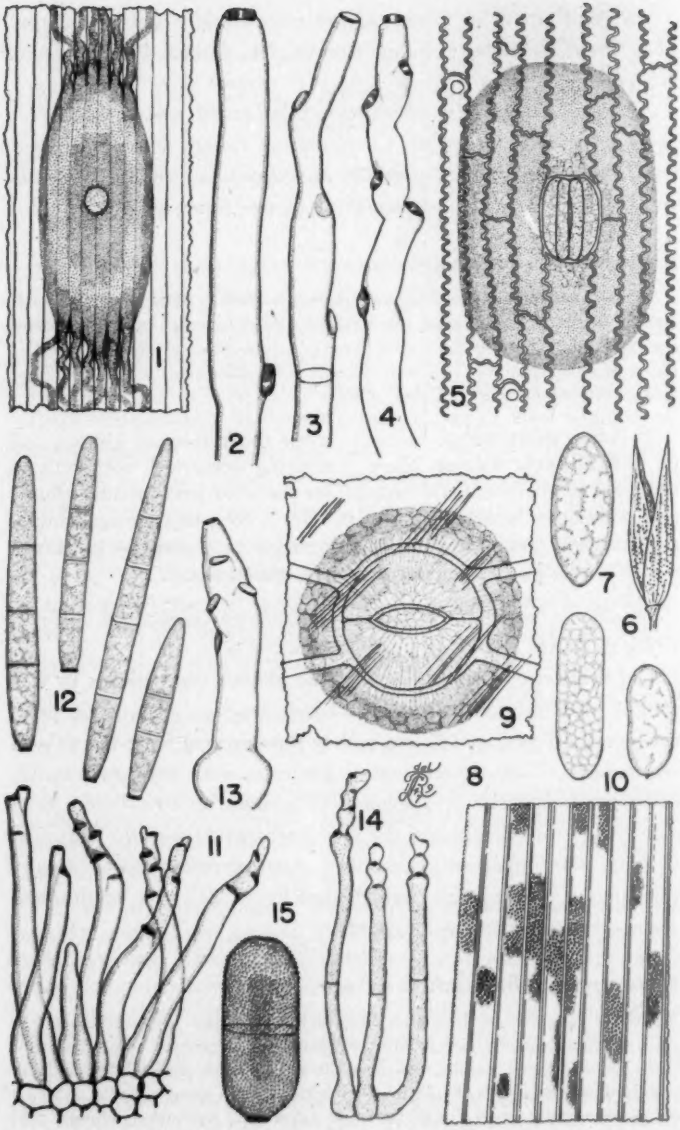
On *Avena sativa* L., Woodford, Woodford County, Ill., July 1, 1938, Acc. No. *29926* (**type**). (FIGS. 5–7.)

Oat spikelets, when attacked on the glumes and pedicels by this fungus, develop poorly and bear small grains. Also, their pedicels are short and spread less widely than normal from the axis of the panicle. The association of pycnidia with stomata appears constant; hence, the arrangement of stomata in rows along each side of the veins determines the seried arrangement of the pycnidia.

From the imperfectly described *Leptostroma Avenae* Brun., this fungus appears well distinguished by its lack of a longitudinal slit and by its longer, wider spores.

Leptothyrium fimbriatum sp. nov.

Maculis amphigenis, 1–12 mm. diametris, rotundatis, atrocinereo-griseis, zona angusta brunnea circumdatis; pycnothyriis plerumque epiphyllis, subcuticularibus, nigris, nitidis, 60–150 μ diametris, saepe ambitu fimbriatis et hyphis aliformibus subcuticularibus connexis; centro poro rotundo, 6.5–13 μ diametro apertis; sporis ovalibus usque sphaericis, hyalinis, continuis, 6.5–12 × 8–13.5 μ.



FIGS. 1–15. Characteristics of Illinois fungi.

Spots amphigenous, 1–12 mm. in diameter, round, dark ash-gray, narrowly brown-bordered; pycnothyria for the most part epiphyllous, occasionally hypophyllous, subcuticular, round, black, shining, 60–150 μ in diameter, appearing parenchymatic but their outermost layer consisting of aliform cells, often more or less fimbriate at the margins and frequently connected by proliferating, subcuticular strands of aliform mycelium; pore present, central, 6.5–13 μ in diameter; spores oval to spherical, hyaline, nonseptate, 8–12 × 6.5–13.5 μ.

On leaves of *Smilax hispida* Muhl., Topeka, Mason County, Ill., October 20, 1937, Acc. No. *30050* (**type**); on leaves of *Smilax rotundifolia* L., Sparta, Randolph County, Ill., July 28, 1926, Acc. No. *20945*.

In 1933 the writer (**9**) called attention to the second collection cited above, assigning it tentatively to *Phyllosticta Smilacis* Dearn., and in figure 7 illustrated the fringe of aliform mycelium often found surrounding the margins of the pycnothyria. In the first collection cited above, which contains the more abundant material, is obviously the more mature, and, therefore, is the better suited for specification as the type specimen, the marginal aliform mycelium often extends, at irregular points, as simple or branched, subcuticular, flat strands of aliform hyphae that stretch from one pycnothyrium to another. As these strands never appear to meet between pycnothyria, it seems clear that pycnothyria develop upon them at intervals as they grow in length.

This species, being foliicolous, is not likely to be confused with *Leptothyrium Smilacis* Dearn., which, as Dearness (**1**) recorded, is caulicolous and has oblong pycnidia up to 2 mm. long and oblong spores measuring 6–12 × 3 μ.

Leptostromella Solani sp. nov.

Pycnidiis in caulibus in maculis brunneolis 1–5 mm. longis, dimidiatis, nigris, nitidis, subcuticularibus, anguste ellipticis, 300 μ vel ultra longis, 120–150 μ latis; mycelio clypei aliformi; loculo 120–150 × 90–110 μ; poro centro rotundo apertis, postea longitudinaliter disruptis; sporis acicularibus usque fusiformibus, continuis, hyalinis, utrinque acutis, rectis curvulisve, 11–19 × 1.5 μ.

Caulicolous in pale brown areas surrounding stems, extending 1–5 cm. in length, and limited by narrow, dark brown, slightly raised margins; pycnidia dimidiate, black, shining, subcuticular,

narrowly elliptical, 300 μ or more long, 120–150 μ wide; mycelium of the clypeus aliform; locule 120–150 × 90–110 μ; opening at first by a central, round pore, later by a lengthwise split; spores acicular to spindle-shaped, nonseptate, hyaline, acute at both ends, straight or variously curved, 11–19 × 1.5 μ.

On stems of *Solanum carolinense* L., LeRoy, McLean County, Ill., October 11, 1943, Acc. No. *30051* (**type**). (FIG. 1.)

Although the outermost layer (called clypeus in the above description) of the pycnidium is composed of cells clearly recognizable as of aliform origin, the ends of the pycnidium usually have extending from them several subcuticular hyphae, dark brown near the pycnidium but fading to hyaline at a distance from the pycnidium, that show no aliform branching. Evidence of the radiate character of the pycnidium is to be found, therefore, only in the cells of the clypeus.

ACTINOPELTE DRYINA (Sacc.) Hoehn.

Limber and Cash (**3**) have carefully considered the morphology, taxonomy and synonymy of this fungus and have given an extended and detailed description of it as they observed it on a variety of hosts. They did not, however, attempt to obtain for examination three species described by the writer, although they listed these species as questionable synonyms. The names of these species, published as *Actinothyrium gloeosporioides* Tehon (**8**), *Pirostoma Nyssae* Tehon (**8**) and *Leptothyriella Liquidambaris* Tehon & Stout (**10**), each with accompanying figures, are of course to be referred to *Actinopelte* and, considering the broad inclusiveness of Limber and Cash's specific description, to the species *dryina* as well. Hence, the occurrence of *Actinopelte dryina* on *Liquidambar styraciflua* in Illinois as well as Maryland and on *Sassafras officinale* in Illinois as well as New Jersey may be recorded. Although in the Illinois samples on *Nyssa sylvatica* "mature" spores are brown—deeper in color than olivaceous and without a green tint—the same color may be found in "mature" spores on *Quercus* and other hosts. The fine verrucose markings appear clearly only on the more "mature" spores. Hence, there can be little doubt that the Illinois material on *Nyssa* is conspecific with material collected on the same host by Diehl in Virginia, which Limber and Cash examined.

Additional records, place and host, for Illinois are as follows:

On Acer saccharum Marsh.—Buncombe, Johnson County, August 5, 1922 (Acc. No. *29732*) and Kinderhook, Pike County, June 28, 1944 (Acc. No. *30074*). In these samples, a large majority of the fruiting bodies are on the upper leaf surface, toward the periphery of circular spots 8–10 mm. in diameter that are dotted below with *Gloeosporium* acervuli. This habit is like that on Sassafras, where the rhizothyria are found epiphyllous on spots sometimes carrying hypophyllously the acervuli usually called *Gloeosporium affine*. The rhizothyria are small, mostly 60 to 65 μ in diameter, but the spores are of the usual size, measuring 11–13 × 8–9.5 μ. On the same host are specimens taken at Bald Knob, Union County, June 13, 1927 (Acc. No. *29734*), at Carbondale, Jackson County, July 29, 1938 (Acc. No. *29733*), at White City, Macoupin County, August 30, 1937 (Acc. No. *29742*), and at Ozark, Johnson County, July 18, 1931 (Acc. No. *29747*).

On Cercis canadensis L.—Mounds, Pulaski County, August 10, 1926 (Acc. No. *29737*). On this host the spots range from 3 to 5 mm. in diameter, are round or somewhat irregular, light brown with narrow purple brown borders, and obviously had their origins in minute hypophyllous galls resulting from the egg-laying activities of an insect. On this host, as on *Acer saccharum*, *Actinopelte dryina* appears to be a secondary invader.

On Fraxinus americana L.—Dongola, Union County, August 10, 1929 (Acc. No. *29736*); Boskydell, Jackson County, July 23, 1923 (Acc. No. *5562*); Eagle Mountain, Saline County, July 14, 1922 (Acc. No. *29741*); and Balcom, Union County, August 11, 1934 (Acc. No. *29744*). Spots range from 4 to 15 mm. in diameter, are tan with narrow purple borders above and tan without borders below. On these samples, rhizothyria are exceptionally abundant and occur on both surfaces of most spots, epiphyllously only in a few spots.

On Fraxinus tomentosa Michx. f.—Dongola, Union County, August 11, 1927 (Acc. No. *29738*).

On Sassafras albidum (Nutt.) Nees.—Fountain Bluff, Jackson County, June 20, 1924 (Acc. No. *17547*); on var. *molle (Raf.) Fern.*—Marlow, Jefferson County, September 7, 1926 (Acc. No. *29739*). In this sample the spots bear no acervuli of *Gloeosporium* on the underside but, like the sample on *Cercis canadensis*, contain small, centrally located insect galls. The rhizothyria are exclusively epiphyllous. Also at Salem, Marion County, August 13, 1926 (Acc. No. *29745*), and Mounds, Pulaski County, August 10, 1926 (Acc. No. *29748*).

On Ulmus alata Michx.—Sanburn, Johnson County, June 15, 1927 (Acc. No. *29740*). Spots are darker brown on this host than on most others and small, reaching 3 to 4 mm. in diameter. The rhizothyria are more numerous on the upper, but frequent also on the lower, surfaces of the spots.

On Liquidambar styraciflua L.—Elizabethtown, Hardin County, June 17, 1927 (Acc. No. *29743*).

On Carya ovata (Mill.) K. Koch.—Simpson, Johnson County, July 11, 1929 (Acc. No. *29746*).

On Quercus as follows: On unidentifiable *Quercus* seedling leaves at Mounds, Pulaski County, August 10, 1926 (Acc. No. *29749*); on *Quercus*, leaf fragments not identifiable to species, in Pope County, August 3, 1922

(Acc. No. *11752*), and Dongola, Union County, August 12, 1922 (Acc. No. *995*); on *Q. imbricaria* Michx. at West Union, Clark County, September 27, 1930 (Acc. No. *29750*), Salem, Marion County, August 13, 1926 (Acc. No. *29751*), Kinmundy, Marion County, June 14, 1922 (Acc. No. *29752*), Red Bud, Randolph County, August 24, 1926 (Acc. No. *27753*), Brownsville, White County, July 11, 1922 (Acc. No. *5164*), and Parker, Johnson County, July 27, 1922 (Acc. No. *1880*). On this host rhizothyria may be found in spots ranging from 3 mm. in diameter to one third or more of the size of the leaf and are as abundant on the lower sides as on the upper sides of the spots. On *Q. marilandica* Muench. at Pinckneyville, Perry County, October 27, 1927 (Acc. No. *29754*), Flora, Clay County, July 23, 1927 (Acc. No. *29755*), and Edwardsville, Madison County, June 15, 1944 (Acc. No. *30075*); on *Q. palustris* Muench. at West Union, Clark County, September 27, 1930 (Acc. No. *29735*), Reevesville, Johnson County, July 27, 1922 (Acc. No. *29756*), Makanda, Jackson County, August 19, 1922 (Acc. No. *29757*), Pulaski County, August 9, 1922 (Acc. No. *8553*), Reevesville, Johnson County, July 27, 1922 (Acc. No. *1833*), and Hagerstown, Fayette County, September 1, 1944 (Acc. No. *30127*); on *Q. stellata* Wang. at Lusk, Pope County, June 16, 1927 (Acc. No. *29758*), and Golconda, Pope County, July 28, 1922 (Acc. No. *1000*); on *Q. velutina* Lam. at Boskydell, Jackson County, July 23, 1923 (Acc. No. *6423*), Renault, Monroe County, July 20, 1923 (Acc. No. *10221*), and Fountain Bluff, Jackson County, June 20, 1924 (Acc. No. *16125*); *Q. borealis maxima* (Marsh.) Ashe at Fountain Bluff, Jackson County, June 20, 1924 (Acc. No. *5541*), Goreville, Johnson County, June 22, 1924 (Acc. No. *3223*), Makanda, Jackson County, August 18, 1922 (Acc. No. *11134*), Tunnel Hill, Johnson County, July 25, 1922 (Acc. No. *2925*), and Seymour, Champaign County, October 15, 1925 (Acc. No. *9282*); on *Q. alba* L. at Albion, Edwards County, June 13, 1944 (Acc. No. *30073*).

On Rhus Toxicodendron L.—At Sandoval, Marion County, June 6, 1944 (Acc. No. *30072*). On the one sample rhyzothyria occur epiphyllously only in spots associated closely with spots containing pycnidia of *Phyllosticta rhoicola* Ell. & Everh. As the two were not observed in the same spot, it is not clear that the *Actinopelte* follows the *Phyllosticta*.

Stictopatella Iridis sp. nov.

Maculis atro-griseis, nigrescentibus, nervis foliorum limitatis, 1 mm. usque 1 cm. longis, subinde confluentibus; pycnidiis numerosis, amphigenis, patelliformibus, rotundatis usque late ellipticis, 55–90 × 60–125 μ, sub stomatibus sitis, patelliformibus; sporophoris numerosis, hyalinis, acicularibus, apice acutis, 1.5 μ latis, usque 15 μ longis, simplicibus; sporulis hyalinis, continuis, oblongis, utrinque rotundatis, 5.5–10 × 2.2–3.3 μ.

Spots dark gray, becoming black, on one or both leaf surfaces, limited laterally by veins, 1 mm. to 1 cm. long or much longer by confluence; pycnidia numerous, amphigenous, cup-shaped, circular to broadly elliptical, 55–90 × 60–125 μ, located beneath stomata, often contiguous but remaining distinct, cup-shaped to patelliform, without a cover, brown, pseudoparenchymatous; sporophores very

numerous, hyaline, slender, pointed, 1.5 μ wide, up to 15 μ long, simple; spores hyaline, nonseptate, oblong, ends rounded, 5.5–10 × 2.2–3.3 μ.

On leaves of *Iris Shrevei* Small, Urbana, Champaign County, Ill., June 11, 1947 (com. R. A. Evers), Acc. No. *30048* (**type**). (FIGS. 8–10.)

Casual examination of this fungus might result in its placement in *Gloeosporium*. It is, however, truly patelliform, having a distinct but shallow pseudoparenchymatous cup two or three cells thick within which conidiophores arise as in a pycnidium. Apparently no cover is ever developed by the pycnidium, which invariably develops in a substomatal cavity. The long, slender sporophores converge from the inner wall of the cup toward the stomatal opening, through which the spores, cut off one at a time from the tips of the conidiophores, escape.

As a result of reexamination of *Phyllosticta destructiva* var. *Evonymi* Desm., von Hoehnel (2, p. 166) proposed the genus *Stictopatella*, with *S. Evonymi* (Desm.) v. Hoeh. as the first species. His placement and characterization of this genus, translated, was as follows:

Patelloideae–patellatae: pycnidia disc shaped, developed beneath the epidermis, more or less erumpent; basal layer thin, indefinitely cellular; cover thin, consisting of fine, slender cells; conidiophores basal, simple, crowded, parallel; conidia terminal, one-celled, hyaline, rotund or elongate; leafspot inhabitant.

Although our iris-inhabiting fungus lacks a pycnidial cover and does not become erumpent, it appears in all other respects so closely congeneric with Hoehnel's *Stictopatella* as to merit assignment in that genus.

Asperisporium Acori sp. nov.

In foliis emortuis et marginibus emortuis foliorum vivorum; conidiophoris erectis, simplicibus, solitariis seu plerumque 2 usque 30 in caespitibus congestis laxis emergentibus per stomates, brunneis, eseptatis seu quoque sepimenti uno medio vel infra locato divisis, basim nonnihil inflatis sed raro bullatis, levibus, diametro similibus, at apicem initio rotundatis demum contortis et nodulosis, cum uno minus saepe duobus hilis minutis, fuscis, protrudentibus ad apicem et quemque nodum, 4–8 μ crassis, 30 μ (conidium primum gignentibus) usque 120 μ longis; conidiis acrogenis, plerumque non

sed interdum catenulatis, echinulatis, brunneis, typice 1-septatis, non constrictis, seu quoque eseptatis vel 2- et 3-septatis, oblongis, utrinque rotundatis, basim hilo conspicuo fusco protrudenti ornatis, typice 21 × 9.5 μ seu quoque ab 14 × 5.5 μ usque 30 × 13.5 μ an continuis, 2-, vel 3-septatis.

Inhabiting dead leaves and dead tissue on the margins of living leaves; conidiophores erect, simple, single or for the most part 2 to 30 or more crowded in loose fascicles that emerge through stomata, light brown, nonseptate or with one septum located at about the middle or below, at the base somewhat but not usually bulbously enlarged, smooth, of quite uniform diameter, at the tip bluntly rounded at first but becoming contorted and nodulose following production of successive spores, with one, less often two, small, dark, protruding geniscars at the apex and on each of the nodal shoulders, 4–8 μ wide, from 30 μ (when the first spore may be borne) to 120 μ long; spores acrogenous, not usually but occasionally catenate, conspicuously echinulate, brown and typically of the same tint as the conidiophores, typically one-septate but also nonseptate and with two and three septa, not constricted at the septa, oblong, with rounded ends and at the base a conspicuous, dark, protruding geniscar, typically 21 × 9.5 μ but ranging from 14 × 5.5 μ to 30 × 13.5 μ for 3- and 4-celled spores.

On *Acorus Calamus* L., Urbana, Champaign County, Ill., June 17, 1926, Acc. No. *30124* (**type**). (FIGS. 14, 15.)

The genus *Asperisporium,* established by Maublanc (**4**, **5**), is based, as to type species, on *A. Caricae* Maubl. (*Cercospora Caricae* Speg., *Scolecotrichum Caricae* Ell. & Everh., *Epiclinum Cumminsii* Massee, *Pucciniopsis Caricae* Earle) and, as to accompanying species, on the morphological agreement of *Fusicladium Peucedani* Ell. & Holw., *Scolecotrichum Alatroemeriae* Allesch., and *S. punctulatum* Tracy & Earle with the fungus on *Carica,* all of which Maublanc transferred to *Asperisporium.*

Upon comparison with the original description and illustrations, the Illinois fungus on *Acorus* proves congeneric with Maublanc's *Carica* fungus but differs specifically in lacking a highly developed stroma, in having more slender, longer, spreading conidiophores and generally larger spores densely covered with minute, conical echinulations rather than irregular, thick, platelike verrucae.

Cercospora difformis sp. nov.

Maculis rotundatis, 0.5–2 mm. diametris, albis, zona angusta, lutea elevata definitis, demum dejicientibus; caespitulis amphigenis sed plerique epiphyllis,

tuberculis parvis, raris, cellarum brunnearum compositis oriundis, unum conidiophorum usque 20, plerumque 12 vel 14, continentibus, cum conidiophoris externis communiter procumbentibus et eis internis rigide erectis et unam densam fasciam vel duas formantibus; conidiophoris brunneis, diffor-mibus; eis procumbentibus in partes tres, basilare 20–30 μ longa, 3 vel 4 cicatrices sporarum gerente, mediata nonnumquam 100 μ longa cicatricem nullam gerente, et distante usque 125 μ longa, 1 usque 8 cicatrices gerente, tota conidiophora parte distante excepta regulariter intervalibus 20–26 μ septata, usque 320 μ longa, 4–5.5 μ crassa; eis erectis 1- vel 2-septatis prope basim et unam cicatricem sporae usque 5 gerentibus, 45–110 μ longis, 4.5–6 μ crassis, minus tenuibus quam quae sunt procumbentes; sporis hyalinis, acicularibus, base ad apicem pariter tenuatim, prope basim intervalibus 10–12 μ septatis, ultra septis imperspicuis, 80–250 μ longis, 2.5–4 μ crassis.

Spots round, 0.5 to 2 mm. in diameter, white, with narrow, raised, yellow to tan borders, eventually falling out; cespitules amphigenous but more numerous on the upper surface, arising from small, loose tubercles of brown cells, consisting of 1 to 20, commonly 12 to 14, conidiophores, the outer conidiophores usually procumbent, the inner ones standing stiffly erect in one or two tight fascicles; conidiophores brown, of two types, a procumbent kind consisting of a basal section 20 to 30 μ long with 3 or 4 spore scars, a central section sometimes 100 μ long without spore scars, and a distal section up to 125 μ long bearing one to 8 spore scars, the whole conidiophore regularly septate at intervals of 20 to 26 μ except in the terminal section, up to 320 μ long, 4–5.5 μ wide, and an upright kind with one, sometimes two, septa very near the base and one to 5 spore scars, 45–110 μ long, 4.5–6 μ wide, less slender than the procumbent kind; spores hyaline, acicular, tapering regularly from base to tip, septate in the basal part at intervals of 10 to 12 μ, septa in the fine tip indistinct, 80–250 μ long, 2.5–4 μ wide.

On leaves of *Viola* (*papilionacea* Pursh?), Vandalia, Fayette County, Ill., October 11, 1944, Acc. No. *30224* (**type**); Abingdon, Knox County, Ill., August 7, 1922, Acc. No. *16200*. On *V. sororia* Willd., Roseville, Warren County, Ill., July 31, 1922, Acc. No. *15567*. (FIG. 3.)

Cercospora sororiae sp. nov.

Maculis majusculis, rotundatis usque ovalibus, 1 cm. vel plus latis aut non limitatis et partem magnam folii destruentibus, supra luteo-brunneis, subtus brunneis; caespitulis numerosissimis, amphigenis sed abundantioribus subtus, e paucis usque 100 sporophoribus efformatis, e tuberculis nigris, densis, stromatoideis plerumque 30–50 μ latis exsurgentibus; conidiophoris pallide brunneis, confertis, erectis, sursum effusis et cum aliquot prominentibus congestis

cicatricibus sporarum, non septatis, non ramulosis, 3–4 × 25–40 μ; sporis hyalinis usque pellucidis fumoso-brunneis, teretibus, apice rotundatis, base cicatricibus externis conspicuis fuscis ornatis, 2- usque 5- plerumque 3-septatis, 3.5–4 × 30–60 μ.

Spots rather large, round to oval, 1 cm. or more wide or unlimited and involving a large part of the leaf, yellow brown above, brown below; cespitules very numerous, amphigenous but much more abundant on the lower leaf surface, consisting of few to 100 or more sporophores rising from black, dense, stroma-like tubercles usually 30 to 50 μ wide; conidiophores pale brown, crowded, erect, spreading above and with several prominent spore scars crowded at the tips, nonseptate, simple, 3–4 × 25–40 μ; spores hyaline to smoky brown, terete, rounded at the apex, bearing at the base conspicuous dark, external scars, two- to five- but for the most part three-septate, 3.5–4 × 30–60 μ.

On leaves of *Viola sororia* Willd., Kinderhook, Pike County, Ill., June 28, 1944, Acc. No. *30126* (**type**). (FIGS. 11, 12.)

The addition, above, of two species of *Cercospora* to the group of eight already described is justifiable on the ground that the new species are distinct and readily separable. Among the older species *C. macrospora* Osterw. is distinguished by the swordlike appendage at the base of the spore, a character aligning it as one of the synonyms of *Centrospora acerina* (Hartig) Newhall (**6**). On the basis of extant descriptions, it and the other species can be rather clearly sorted under the system proposed by Solheim (**7**), as follows:

I. Each spore bearing a slender, retrorsely directed appendage at its base (*Cercospora macrospora* Osterw.) . . *Centrospora acerina* (Hartig) Newh.
II. Spores without such appendages. *Cercospora.*
  A. Only internal mycelium present.
    1. Conidiophores simple.
      a. Stroma tuberculate, spores cylindrical (Solheim's Section III); conidiophores short, nonseptate (FIG. 11); spots unlimited . . . . . *C. sororiae*
      a. Stroma loose or absent.
        b. Spores acicular-obclavate (Solheim's Section IV).
          c. Conidiophores all more or less erect and alike.
            d. Conidiophores short; spore scars several, often causing geniculations (FIG. 4) . . *C. Violae* Sacc.
            d. Conidiophores long, expanded toward the tips; spore scars few, widely separated (FIG. 2) *C. Violae-tricoloris* Br. & Cav.

c. Conidiophores of 2 kinds, procumbent and erect; the procumbent very long, septate, with spore scars at base and tip; the erect shorter, closely fascicled, with spore scars at tip .....*C. difformis*

b. Spores cylindrical (Solheim's Section VI).

e. Conidiophores repent, very long, hypophyllous; spores 5–6.5 μ wide .....*C. lilacina* Bres.

e. Conidiophores erect, shorter, amphigenous; spores more slender, up to 4.5 μ wide.

f. Conidiophores very short, nonseptate, with spore scars crowded near the tips (FIG. 13); spots unlimited .....*C. granuliformis* Ell. & Holw.

f. Conidiophores longer, septate; spots circular, limited .....*C. Violae-sylvaticae* Oud.

2. Conidiophores alternately branched, stroma loose, spores cylindrical (Solheim's Section X) .....*C. murina* Ell. & Kellerm.

B. Both external and internal mycelium present, conidiophores simple, stroma loose, spores abruptly obclavate (Solheim's Section XVI); spots small, limited .....*C. Ii* Trail.

Of the old species listed in the above key, we have specimens that may be assorted as given below:

C. granuliformis Ell. & Holw. on *Viola* (*papilionacea* Pursh?): America, Pulaski County, June 18, 1941, Acc. No. *28309;* Bement, Piatt County, July 6, 1925, Acc. No. *1576;* Marshall, Clark County, July 22, 1941, Acc. No. *28336.*—This species is readily recognized by the extensive leaf area which it kills, its small, compact cespitules, the notable distribution of cespitules along the leaf veins, and its very short conidiophores which are bulbous at the base and bear one to several closely crowded spore scars toward the tip (FIG. 13). It and the new *C. sororiae* described above are very similar, differing chiefly in size and compactness of stroma and conidiophores (FIGS. 11, 12).

C. Violae Sacc. on *Viola* (*papilionacea* Pursh?): Bement, Piatt County, July 6, 1925, Acc. No. *4087;* Clinton, DeWitt County. July 21, 1925, Acc. No. *11986;* Kansas, Edgar County, July 17, 1925, Acc. No. *3952;* Mackinaw, Tazewell County, June 6, 1923, Acc. No. *17411;* Mahomet, Champaign County, August 30, 1921, Acc. No. *10385;* Mason, Effingham County, June 11, 1936, Acc. No. *25484;* Oneida, Knox County, August 9, 1922, Acc. No. *13811;* Peonydale Farm, McDonough County, July 20, 1922, Acc. No. *15775;* Pittsfield, Pike County, July 24, 1925, Acc. No. *8676.*

On *Viola sororia* Willd.: Lewistown, Fulton County, July 12, 1922, Acc. No. *16312;* Thompsonville, Franklin County, July 21, 1922, Acc. No. *2705.*—Characterized by small, round, persistent spots usually 1–2 mm. in diameter, acicular spores, virtual absence of a tubercle, and short conidiophores markedly geniculate and with several spore scars near the tip (FIG. 4), *C. Violae* is apparently the most common species on *Viola* in Illinois. Although Saccardo's description gives the spore length as 150–200 μ, spores 150 μ long are uncommon in Illinois samples; the usual spore lengths range between 55 and 135 μ.

C. Violae-tricoloris Br. & Cav. on *Viola* (*missouriensis* Greene?): Lawrenceville, Lawrence County, June 9, 1942, Acc. No. *28687.*—The single specimen referred to this species is so placed with hesitation. Although the spots, 3 to 4 mm. in diameter, are larger than those of other "round spot" species and the conidiophores and spores agree well as to measurements with the description, the conidiophore apex is distinctive in appearance (FIG. 2). When the first spore is seen on the tip of the conidiophore, the tip appears swollen or inflated. After discharge of the spore, growth of the conidiophore continues at this larger diameter, and spore scars, widely spaced, become prominent, dark excrescences on the side of the hypha, causing no indentations or geniculations.

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EXPLANATION OF FIGURES

FIG. 1. *Leptostromella Solani,* surface view of a pycnidium. FIG. 2. *Cercospora Violae-tricoloris,* terminal part of a conidiophore. FIG. 3. *Cercospora difformis,* terminal part of a procumbent conidiophore. FIG. 4. *Cercospora Violae,* terminal part of a conidiophore. FIGS. 5–7. *Leptothyrium Avenae:* 5, surface view of a pycnidium centered beneath a stoma; 6, habitat sketch; 7, a spore. FIGS. 8–10. *Stictopatella Iridis:* 8, habitat sketch; 9, pycnidium beneath a stoma; 10, two spores. FIGS. 11–12. *Cercospora sororiae:* 11, group of conidiophores; 12, four spores of different sizes. FIG. 13. *Cercospora granuliformis,* conidiophore. FIGS. 14–15. *Asperisporium Acori:* 14, three conidiophores; 15, a spore.

KERATINOPHILIC CHYTRIDS. III. RHIZOPHYDIUM NODULOSUM SP. NOV.

JOHN S. KARLING[1]

(WITH 20 FIGURES)

In previous publications the author (3, 5) reported on the use of keratinized tissues such as hair, skin, hoofs, horns, nails and feathers as substrata for isolating specialized types of chytrids, and described two new species which appear to be limited in occurrence to tissues containing keratin. The present contribution deals with a third new species isolated on human hair from muck soil from Van Cortlandt Park, New York City, and grown on other keratinized substrata in filtered, sterile soil extract. This species is characterized by ectobiotic sporangia and branched endobiotic rhizoids which distinguish it as a species of *Rhizophydium*. More specifically it is distinguishable by sporangia with a large number of prominent exit papillae which give them a strikingly gibbose, angular or nodular appearance and shape. On the basis of this and other structural characteristics as well as the fact that it grows only on keratinized substrata in nature, it is distinct from the other known species of this genus. Accordingly, it is diagnosed as a new species and named *R. nodulosum* because of the characteristic shape and appearance of the sporangia.

Rhizophydium nodulosum sp. nov.

Fungus saprophyticus. Sporangiis hyalinis, laevibus, plerumque angularibus, irregularibus, 65 μ diam., aut ovalibus, 10–25 × 15–50 μ diam., aut sphaericis, 10–35 μ, oblongis vel pyriformibus, 1–15 papillatis, 4–8 × 8–15 μ diam. Zoosporis sphaericis, 2.8–3.2 μ diam., globulo refractivo 0.4–0.8 μ diam., flagello 15–18 μ longo. Hyphis rhizomorphis ramosis, 30–270 μ longis. Sporis perdurantibus ignotis.

Sporangia hyaline, smooth, predominantly angular or nodular, up to 65 μ in diam., oval, 10–25 × 15–50 μ, spherical, 10–35 μ, oblong, pyriform or irregular with 1–15 prominent exit papillae,

[1] The author is very grateful to T. K. Just of the Chicago Natural History Museum for the Latin diagnoses.

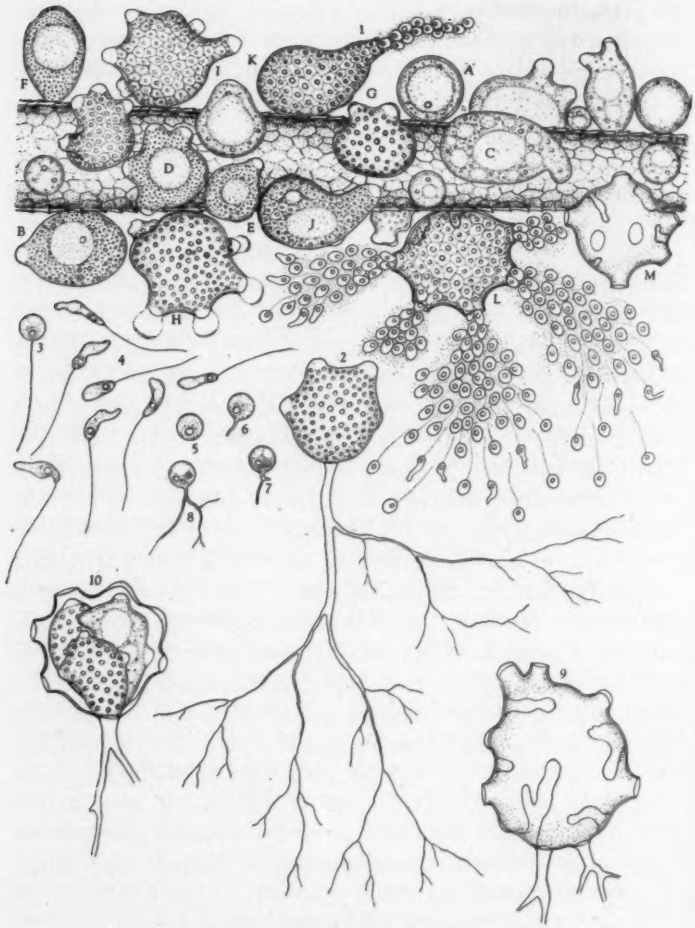
4–8 μ high by 8–15 μ broad at the base. Zoospores spherical, 2.8–3.2 μ, with a minute refractive globule, 0.4–0.8 μ diam.; flagellum 15–18 μ long. Rhizoids usually well developed but sparingly branched, arising from one to several points on the base of the sporangium, main axes up to 7 μ in diam., branches extending for distances of 30–270 μ. Resting spores unknown.

Saprophytic on dead human hair and other keratinized substrata in muck soil and freshwater, New York City, N. Y.

This species was first collected on January 30, 1947, when the temperature was very low. In fact, the muck soil was frozen solid at that time and had to be chopped from the ground. After thawing out in a culture dish in the laboratory, the soil was covered with filtered, sterile soil extract and baited with short segments of blond hair. The latter became infected within four days, which indicates that the chytrid develops very rapidly or was already present in the frozen soil in a reproductive stage. It was isolated later in May of the same year in freshwater from the same locality and grown extensively on dead human skin and other keratinized substrata. All attempts to grow it on cellophane, bleached grass leaf segments, chitin and several agar media have failed, which suggests that it may be specialized in its nutritional requirements.

Fundamentally *R. nodulosum* develops in the same manner as other well-known members of *Rhizophydium*, so that it is unnecessary to give a detailed account and illustration of this process. Only the most outstanding stages and structural characteristics will be emphaszied to distinguish this from other species of the genus. As shown in figure 1 bits of floating hair may become very thickly infected and almost covered with sporangia in various stages of development. In some instances infection was almost as great as reported previously (Karling, **5**) for *Rhizophydium keratinophilum* on the same substratum. Because of the thickness and refractive index of the hair, the endobiotic rhizoids are usually invisible, but when the chytrid is grown on thin, relatively transparent skin they are distinctly visible. They may be fairly short and fine or relatively coarse and extensive (FIG. 2), but in all thalli observed so far they were not very richly branched.

As noted in the diagnosis above, the outstanding structural characteristic is the irregular, nodular and gibbose shape of the



FIGS. 1–10. *Rhizophydium nodulosum.*

sporangia, which in most cases is associated with the development of numerous prominent exit papillae. In the gibbose character of the sporangia this species resembles *R. gibbosum* (Zopf, **8**; Karling, **4**) to some degree, but in the latter species, which parasitizes various algae and rotifer eggs, only one exit papilla is formed. In

the young stages of *R. nodulosum* the sporangia are usually oval, slightly elongate to spherical in shape and contain a large central vacuole (FIG. 1A, 1B), but later a number of smaller additional vacuoles appear (FIG. 1C) which give the sporangium of this species a very characteristic internal appearance on hair. At this stage comparatively few conspicuous refractive globules are present in the cytoplasm (FIG. 1C), and the sporangia do not have the typical refringent appearance of most chytrids. The vacuolate condition persists for a long period and appears to be one of the most striking developmental phases. The multivacuolate stage is usually followed by one in which only a single large vacuole is present (FIG. 1D). At the same time the refractive material becomes more highly dispersed so that the protoplasm becomes greyish-granular in appearance (FIG. 1D, 1E, 1F). This appearance also is a striking, visible developmental stage of *R. nodulosum*. It is followed eventually by the coalescence of the refractive material to form the definitive globules of the zoospores (FIG. 1G, 1H, 2), and by cleavage (FIG. 1I, 1G).

In the meantime one to several prominent exit papillae develop which are usually very broad, fairly high, and occasionally elongate like a tube. During deliquescence, the outer wall may sometimes swell to remarkable proportions, as shown in figure 1H. As the tips open the zoospores ooze out in elongate masses (FIG. 1K), become active after a few seconds, and then dart away. Several exit papillae may deliquesce simultaneously so that several streams of zoospores may be emerging from the sporangium (FIG. 1L) at the same time. Zoospores which fail to emerge may often germinate *in situ* and develop into sporangia, as shown in figure 10.

Another common occurrence in *R. nodulosum* is the development of small or large, irregular, simple or branched plugs or ingrowths from the wall of the sporangia. These become particularly conspicuous when the sporangia are empty (FIG. 1M, 9). These ingrowths, however, cannot be regarded as specific because similar ones have been described in species of *Chytriomyces* and other chytrids (Haskins, **2**; Karling, **6**).

So far resting spores have not been observed so that our knowledge of *R. nodulosum* is incomplete.

PARASITE OF RHIZOPHYDIUM NODULOSUM

During the course of this study of *R. nodulosum* its sporangia became infected by another minute chytrid which may prove to be an obligate parasite. At least it did not attack *Chytriomyces hyalinus, C. stellatus, Phlyctorhiza variabilis, R. keratinophilum, Karlingia rosea, Pythium debaryanum,* or *Aphanomyces laevis* which were growing luxuriantly on other substrata in the same cultures at the time of infection. Furthermore, it did not parasitize several species of filamentous and unicellular algae which were also present. For a period of time the infection of *R. nodulosum* was so extensive and severe that most of the thalli were killed. In several cases observed from ten to fifteen parasites were present on one sporangium (FIG. 11). However, the epidemic subsided within three weeks, and thereafter only sporadic infections occurred.

The parasite is characterized by narrowly obpyriform or citriform, oval or elongate, epibiotic operculate sporangia which may be sessile or slightly stalked, by minute finely-branched or unbranched rhizoids, and by minute zoospores. Its epibiotic operculate sporangia and endobiotic simple or branched rhizoids distinguish it either as a species of *Chytriomyces* or *Chytridium,* but inasmuch as its resting spores are unknown its generic position is uncertain. The majority of *Chytridium* species are apophysate and form intramatrical resting spores in the position of the apophysis, whereas species of *Chytriomyces* may be apophysate or non-apophysate and form extramatrical resting spores. Since the parasite of *R. nodulosum* is non-apophysate and has a minute absorbing system it does not seem likely that its resting spores will be formed endobiotically as in most *Chytridium* species. On the other hand, its zoospores do not swarm in a vesicle after emerging from the sporangium as in species of *Chytriomyces.*

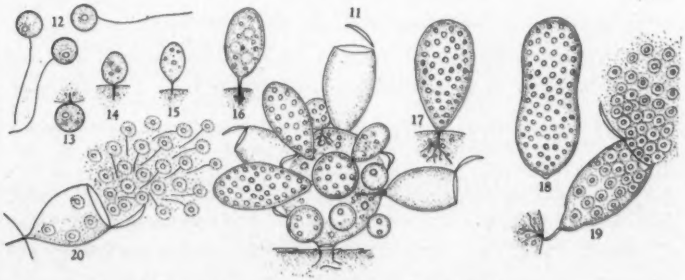
In the shape of the sporangia and the character of the endobiotic absorbing system it resembles somewhat *Chytridium versatile* (Scherffel, 7), *C. versatile* var. *acaulis, C. cocconeidis* (Canter, 1), and other questionable *Chytridium* species which parasitize algae, are non-apophysate, and have minute, branched or unbranched rhizoids. Resting spores are unknown in these *Chytridium* spe-

cies, and here also it is not likely that such spores will be found to occur endobiotically. Like the parasite of *R. nodulosum*, they are questionable members of *Chytridium* and, doubtless, will have to be removed from this genus when it is fully known. Although the parasite of *R. nodulosum* is somewhat similar to the species mentioned above, it differs in host range as well as by several minor structural characteristics, and for this reason it is regarded as a new species. It is included provisionally in the genus *Chytridium* and named *C. Rhizophydii* after the host which it parasitizes.

Chytridium Rhizophydii sp. nov.

Fungus parasiticus. Sporangiis hyalinis, laevibus, citriformibus, ovalibus, 8–15 × 12–22 μ diam., aut obpyriformibus, 10–18 × 22–32 μ diam.; operculo hypocrateriformibus, 8–15 μ diam. Zoosporis sphaericis, 2.2–2.8 μ diam., globulo refractivo hyalino; flagello 12–14 μ longo. Hyphis rhizomorphis eramosis, tenuissimis, 4–10 μ longis. Sporis perdurantibus ignotis.

Sporangia hyaline, smooth, elongately citriform or obpyriform, 10–18 μ × 22–32 μ, oval, 8–16 × 12–22 μ, or elongate; operculum persistent, shallow saucer-shaped, 8–15 μ diam. Zoospores spherical, 2–2.8 μ, with a minute hyaline refractive globule; flagellum 12–14 μ long. Rhizoids unbranched, tapering and needle-like or finely branched, 4–10 μ long. Resting spores unknown.



FIGS. 11–20. *Chytridium Rhizophydii.*

Parasitic on *Rhizophydium nodulosum*, New York, N. Y.

There is nothing unusual in the development of this parasite which distinguishes it sharply from other similar species of *Chytridium*, except that when the zoospores germinate at some distance

from the host the resulting sporangia become stalked as shown in figures 15, 16 and 19. The germinating spore or incipient sporangium is at first spherical (FIG. 13), but it soon elongates and becomes narrowly oval or broadly fusiform (FIG. 15, 16). The mature sporangia are usually of this shape with tapering ends, but in numerous cases they may become narrowly obpyriform (FIG. 17, 19) or even broadly fusiform. The apical operculum is unusually large, and may be only slightly less in diameter than the sporangium (FIG. 20). Particularly noteworthy is the dehiscence of the sporangium. This may often occur with such force that it resembles an explosion. As a large mass of spores is shot out, the operculum is pushed up and back with such speed that its movement is usually invisible. In some cases observed the discharge of the zoospores was so forceful that the sporangium recoiled and broke loose from the attached rhizoid. Figure 19 shows a sporangium in the process of dehiscence with the stalk breaking loose at the surface of the host cell. However, the force required to break the rhizoid is not very great because sporangia may frequently be torn loose in mounting the material for microscopic study (FIG. 18). If such sporangia are mature they will dehisce and discharge zoospores in the usual manner. The discharged zoospores lie quiescent in a slimy matrix for a few seconds and then free themselves and dart away.

As noted before resting spores have not been found in this parasite. Consequently, its life cycle is not fully known, and it is not certain whether it is a species of *Chytridium* or *Chytriomyces*.

SUMMARY

Rhizophydium nodulosum sp. nov. is a keratinophilic saprophyte which was isolated from frozen muck soil and freshwater from Van Cortlandt Park, New York City. It grows readily on keratinized substrata such as hair and skin but not on chitin, cellulose, or various types of nutrient agar media. It is characterized structurally by hyaline, smooth, nodular and angular zoosporangia with several prominent exit papillae which give the sporangia their typical shape and appearance. Resting spores are unknown.

Its sporangia may become heavily parasitized by another small chytrid which is characterized by elongately citriform, obpyriform,

oval or elongate, operculate sporangia and minute branched or unbranched rhizoids. The resting spores of the parasite are unknown so that it is not certain whether this chytrid is a species of *Chytridium* or *Chytriomyces*. Nevertheless, it is included provisionally in *Chytridium* and named *Chytridium Rhizophydii*.

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DESCRIPTION OF FIGURES

FIGS. 1–10. *Rhizophydium nodulosum*. FIG. 1. Segment of human hair infected with 23 thalli in various stages of development. × 860. FIG. 2. Complete thallus from dead human skin. × 900. FIG. 3. Zoospores in active swimming stage. × 1,720. FIG. 4. Amoeboid zoospores. × 1,720. FIGS. 5–8. Zoospores at rest and successive germination stages. × 1,720. FIG. 9. Empty sporangium with secondary sporangia developing *in situ*. × 1,000. FIG. 10. Large irregular empty sporangium with five ingrowths or plugs of material from the wall. × 1,000.

FIGS. 11–20. *Chytridium Rhizophydii*. FIG. 11. Sporangium of *R. nodulosum* parasitized by twelve thalli. × 1,800. FIG. 12. Zoospores in active swimming stage. × 2,500. FIGS. 13–16. Germination stages and development of thalli with unbranched, needle-like rhizoids. × 2,800. FIG. 17. Narrowly obpyriform sporangium with branched rhizoids. × 1,600. FIG. 18. Detached elongate, slightly constricted sporangium. × 1,600. FIG. 19. Discharge of zoospores. × 1,600. FIG. 20. Dissemination of zoospores. × 1,600.

A NEW SPECIES OF ACHLYA

ARTHUR WILLIAM ZIEGLER [1]

(WITH 10 FIGURES)

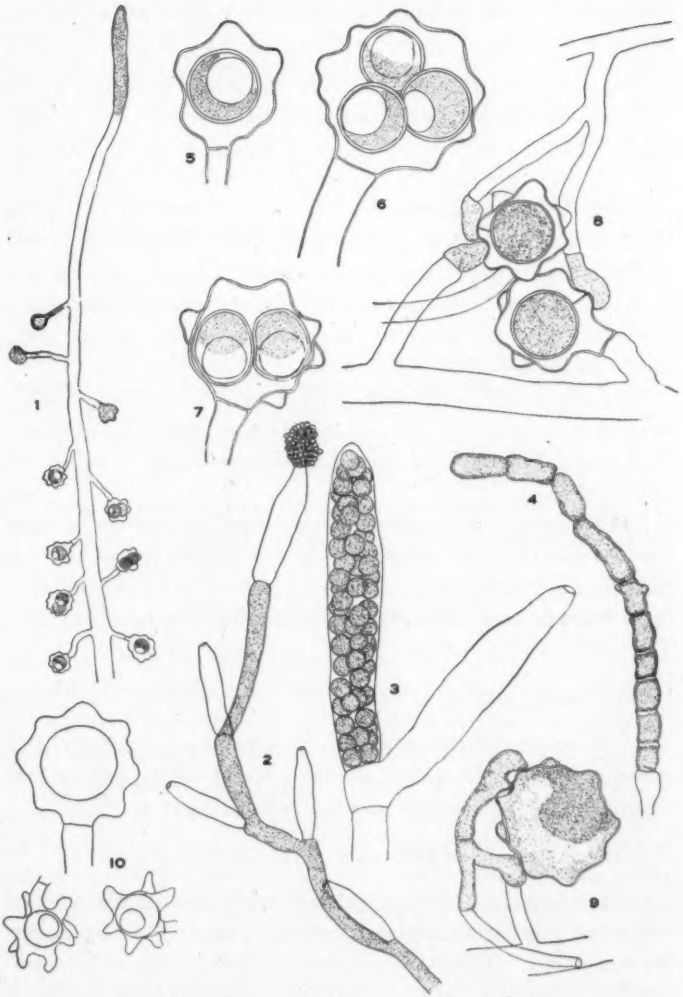
A new species of *Achlya* was found in a water collection made from a ditch near the pump at Kalmia Gardens, Hartsville, South Carolina.

Achlya crenulata n. sp.

Mycelium modice densum, hyphae usque ad 1 cm. longae in seminibus *Cannabis sativae*, diam. culturae plerumque 2–2.5 cm. Sporangia copiosa, longa, cylindrica, in medium ferme 271.4 μ longa et 37.6 μ lata. Ejectio sporae typica generis. Gemmae copiosae, efformatae hypha transformata in seriem cellularum brevium cylindricarum. Oogonia globosa, ex 33.3 μ ad 60 μ in diam., plerumque ferme 48.6 μ, obtusis papillis tecta. Paries terminalis papillarum vel tenuior vel eadem crassitudine. Longitudo caulis oogonii ferme aequalis latitudini oogonii. Ova eccentrica, 1–3, rare 5, 22.2–30.7 μ, plerumque 27.6 μ in diam., saepe ellipsoidea, non complentes oogonium. Antheridia rara, plerumque origine diclina sed interdum androgyna, reperta in ferme uno ex 14 oogoniis, omnino vacua antequam ova matura sunt.

Mycelial growth moderately dense, hyphae reaching a length of 1 centimeter on hemp seed, diameter of culture about 2 to 2.5 cm. Sporangia plentifully formed, long, cylindrical, averaging 271.4 μ by 37.6 μ. Spore discharge typical for the genus. Gemmae plentiful in cultures, formed by the transformation of a hypha into a row of short cylindrical cells. Oogonia spherical, ranging from 33.3–60 μ, mostly about 48.6 μ, covered with blunt crenulate projections. The end wall of the projections may or may not be thinned. Length of oogonial stalk about equal to the width of the oogonium. Eggs eccentric, 1–3, occasionally 5, 22.2–30.7 μ, mostly 27.6 μ in diameter, frequently ellipsoid in shape, not filling the oogonium. Antheridia rarely formed, usually diclinous but occasionally androgynous, occurring on about 1 out of every 14 oogonia, and becoming completely empty before the eggs are mature. Antheridial tube usually present.

[1] Instructor in Botany, University of North Carolina, Chapel Hill, North Carolina. The writer is deeply indebted to Dr. J. N. Couch for his aid and suggestions and to Mrs. C. D. Beers for the Latin description.



FIGS. 1–10. *Achlya crenulata.*

In water on hemp seed bait. Water collected by the author in a ditch near the pump at Kalmia Gardens, Hartsville, South Carolina, February 9, 1947.

This species was thought to be *A. glomerata* when the author first examined it but further study showed that the oogonia were much larger and were not in clustered groups as in *A. glomerata* (1). Figure 10 shows the relative sizes of these two species.

Achlya crenulata differs from *A. radiosa* Maurizio (1) in its blunt papillae and smaller egg-size and from *A. recurva* Cornu (4) in the general shape of the spines, number of eggs, and few antheridia. It can be distinguished from *A. abortiva* Coker and Braxton (2) in its round and not oblong oogonia, shorter stalk, and by all the eggs reaching maturity.

This new species germinates readily after a rest period of six weeks if it is placed in fresh water. A short germ tube is formed with an apical sporangium which discharges its spores in the typical *Achlya* manner.

As previously stated, antheridia occur on about one out of every fourteen oogonia. In order to induce, if possible, a greater antheridial growth, the fungus was grown in several different solutions which favored antheridial branches as used by Kauffman (3). The results were as follows:

1. Mushroom grub.

Mycelium scanty after seven days growth. Long rows of typical gemmae formed. Very few oogonia formed, all typical, no antheridia present. Oogonial and egg measurements as above.

2. 0.05% haemoglobin and 0.2% potassium phosphate.

Growth heavy after seven days, culture 40 mm. in diameter on hemp seed, sporangia abundant, typical gemmae formed abundantly. No sex organs. Twenty-four hours after pure water added, gemmae had germinated to form sporangia. After seventy-two hours abundant oogonia present, antheridia on every third oogonium. Antheridial branches not profusely branched. No change after 120 hours. Oogonial and egg measurements as above.

3. 0.05% haemoglobin and 0.2% potassium nitrate.

After seven days, growth sparse, culture 15 mm. in diameter on hemp seed, few sporangia formed, typical gemmae moderately abundant. No sex organs formed. Twenty-four hours after pure water added gemmae had germinated to form sporangia. After seventy-two hours sex organs formed abundantly. Diclinous antheridia on about every seventh oogonium. Eggs mostly mature. After 120 hours eggs mature—no other change. Oogonial and egg measurements typical for species.

4. 0.05% haemoglobin and 0.1% potassium phosphate with 0.1% potassium sulphate.

After seven days growth was sparse but diameter of colony on hemp seed was 30 mm. Sporangia few, gemmae moderately abundant. No sex organs formed. Twenty-four hours after placing in fresh water one fourth of gemmae had germinated to form sporangia. After seventy-two hours sex organs plentifully formed. Diclinous antheridial branches on every fifth oogonium. After 120 hours no change except eggs mature. Measurements typical for species.

5. 0.05% haemoglobin with 0.1% potassium phosphate and 0.1% sodium chloride.

After seven days growth was heavy. Culture 22 mm. in diameter on hemp seed. Few sporangia formed, gemmae very abundant and could be seen by the naked eye. No sex organs formed. Twenty-four hours after placing in fresh water gemmae germinated to form sporangia. Young oogonia formed with about three-fourths with diclinous, much branched antheridia. After seventy-two hours oogonia abundant and eggs mostly mature. Antheridia with long profuse branches applied to every third oogonium. No change after 120 hours. No change in measurements.

6. 0.05% haemoglobin with 0.1% disodium hydrogen phosphate and 0.1% potassium sulphate.

Growth after seven days moderately abundant. Culture on hemp seed 35 mm. in diameter. Sporangia few, gemmae moder-

ately abundant. No sex organs formed. After twenty-four hours in fresh water one half of the gemmae had germinated to form sporangia. After seventy-two hours no change. After 120 hours a few young oogonia were formed but no antheridia present. Measurements typical.

7. 0.05% leucin.

After seven days growth was moderately abundant. Diameter of culture on hemp seed 30 mm. No sporangia formed and few gemmae. No sex organs. Twenty-four hours after placing in fresh water gemmae formed sporangia. After 72 hours a few young oogonia formed. Antheridial branches profuse but only applied to every other oogonium. After 120 hours eggs mature, antheridial branches formed profusely although not as profusely as in number nine. Egg and oogonium measurements typical.

8. 0.05% leucin plus 0.1% potassium phosphate.

Growth after seven days heavy. Diameter of culture on hemp seed 25 mm. Sporangia very abundant. Gemmae abundant. No sex organs. After twenty-four hours in fresh water all gemmae had germinated to form sporangia. After seventy-two hours young oogonia formed abundantly. Antheridia found on about every twelfth oogonium. After 120 hours eggs mature—no further change. Measurements typical.

9. 0.05% leucin plus 0.1% calcium phosphate.

Growth after seven days sparse, hyphae translucent. Culture on hemp seed 10 mm. in diameter. No sporangia or gemmae formed. No sex organs. Width of hyphae small. Twenty-four hours after placing in fresh water, no change. After seventy-two hours no change. After 120 hours oogonia with eggs moderately abundant with best production of diclinous antheridia seen in any solution. Antheridia on all oogonia. Antheridial branches long, twining and branched. Measurements typical.

SUMMARY

A new species of *Achlya*, *A. crenulata*, is described. It differs primarily from other eccentric species of *Achlya* in that its spheri-

cal oogonia are covered with blunt crenulate projections. The fungus was grown in several media: 0.05% haemoglobin with 0.2% potassium phosphate gave the best vegetative growth. A solution of 0.05% leucin with 0.1% calcium phosphate restricted the vegetative growth but after 120 hours in fresh water antheridia were more profusely formed than in any other solution.

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EXPLANATION OF FIGURES

FIG. 1. Habit of growth, × 178. FIG. 2. Arrangement of sporangia, × 178. FIG. 3. Sporangium about to discharge its spores, × 1,128. FIG. 4. Typical group of gemmae, × 178. FIGS. 5, 6, 7. Oogonia with eggs, × 1,128. FIG. 8. Oogonia showing diclinous antheridia and antheridial tubes, × 1,128. FIG. 9. Immature oogonium showing egg formation and diclinous antheridia, × 1,128. FIG. 10. Outline drawings of *A. crenulata* (above), and *A. glomerata* (below).

HELMINTHOSPORIUM PORTULACAE A NEW PATHOGEN OF PORTULACA OLERACEA L.

WM. E. RADER

(WITH 1 FIGURE)

Portulaca oleracea L. or purslane is a well known weed distributed throughout the temperate United States. It occurs in great abundance in the muckland areas of New York State which are devoted largely to the production of vegetable crops. Purslane is now controlled by pulling and removing the plants from the fields by hand. Because of the rapidity with which this weed reestablishes itself from pieces of stems and the large numbers of seeds produced, its eradication involves considerable time and expense each year.

During 1944 investigations were conducted to determine the possibility of controlling this weed in the field by means of artificially disseminating the fungus *Dichotomophthora portulacae* Mehrlich and Fitzpatrick.[1] Near the test plots at Watkins Glen, New York, another fungus was found attacking the purslane. During 1945 this same fungus was found killing purslane in widely separated areas in New York State. Isolations from the diseased plants have consistently yielded a fungus which is believed to be new.

Greenhouse and field tests with *Dichotomophthora portulacae* and this new pathogen indicate that under the dry conditions which prevail in New York State during the summer these fungi will be of little value in controlling this weed. However, because of the possibility of using either of these fungi for the biological control of *Portulaca oleracea* in regions of high rainfall and humidity, this new disease together with its pathogen are described.

[1] Mehrlich, F. P., and H. M. Fitzpatrick. *Dichotomophthora portulacae* a pathogene of *Portulaca oleracea*. Mycologia **27** (5) : 543–550. 1935.

DESCRIPTION OF THE DISEASE

Symptoms appear in two to five days on plants artificially inoculated with spore suspensions. The brownish, watersoaked lesions which appear along the stem may involve the whole branch or only isolated areas. The smaller lesions spread rapidly and soon cover all of the stem. The diseased areas change to a dark brown color and finally become black. Conidia of the fungus are produced in large numbers on the blackened areas on the stems and leaves.

MORPHOLOGY OF THE PATHOGEN

The brown septate hyphae of the pathogen are 6 to 7.5 μ in diameter, and ramify intercellularly throughout all of the suscept tissues.

TABLE 1

PERCENTAGE DISTRIBUTION OF CONIDIAL TYPES OF *Helminthosporium portulacae* OCCURRING ON *Portulaca oleracea* AND POTATO DEXTROSE AGAR [1]

Spore Class	*P. oleracea*	Potato Dextrose Agar
3-celled	2.35	2.63
4-celled	1.18	5.26
5-celled	2.35	2.63
6-celled	3.53	11.84
7-celled	11.76	15.79
8-celled	9.41	19.74
9-celled	18.82	23.68
10-celled	29.41	9.30
11-celled	9.41	7.89
12-celled	4.71	—
13-celled	5.88	—
14-celled	1.18	—

[1] Based on measurements of 250 spores.

In culture on potato dextrose agar the mycelium is at first hyaline, sparse and appressed to the medium. The colony soon becomes olivaceous brown and then black, profusely dotted with small bulbils. The hyphae in culture are 5–7 μ in diameter, septate and anastomose sparingly.

The conidia are brown, straight or slightly curved, elongate, rounded at the ends, 3–14 (mostly 9–10) celled (Table 1) and average 110.2 × 12.8 μ (Table 2).

The conidia are borne in fascicles of three to ten on brown, septate conidiophores 40–200 μ long. The conidiophores are sim-

TABLE 2

SIZE OF CONIDIA IN MICRONS OF *Helminthosporium portulacae*[1]

Spore Class	Length	Width
3-celled	36.6	10.0
4-celled	43.3	10.4
5-celled	65.6	11.9
6-celled	82.1	11.7
7-celled	94.9	12.5
8-celled	112.9	13.8
9-celled	115.9	13.7
10-celled	141.3	13.5
11-celled	141.2	13.2
12-celled	171.0	13.1
13-celled	173.5	14.1
14-celled	183.2	14.7

[1] Based on a mean of at least 15 spores in each class.

ple or occasionally branched and are produced singly or in clusters on the stems of *Portulaca* sp. or on culture media.

The bulbils are black, globoid or irregular in shape and 53–276 μ (ave. 114.3 μ) in diameter, black without, hyaline to gray within.

No perfect stage of the fungus has been found. The characters detailed above appear to place this organism in the genus *Helminthosporium*. A search of the literature reveals no species of the genus parasitic on any of the species of *Portulacaceae*. Neither do the characters of the pathogen approach closely any of the described species of *Helminthosporium*. A new species is therefore established, the formal description as follows:

Helminthosporium portulacae sp. nov.

Mycelium septatum, primum hyalinum, actutum olivaceo fuscum vel nigro fuscum evadans. Conidiophoria fusca, simplicia, in substrato singulatim aut gregatim portata, 40–200 μ longitudine × 5–7 μ diametro. Cellae terminales conidiophorium levidensis contortae, ferentes 3–10 conidia fusca, elongata, recta vel levidensis curvata, in terminis orbiculata, cellis 3–14, plerumque 9 aut 10, 29.3–183.3 × 9.1–16.5 μ (medio 110.2 × 12.8 μ). Bulbiles abundantes, minuti, extra nigri, intus hyalini vel cani, forma sphaerici vel irregulares, 53–276 μ (medio 114.3 μ) diametro.

Domicilium: biogenum in *Portulaca oleracea* L.

Mycelium septate, at first hyaline, soon becoming olivaceous brown to dark brown. Conidiophores brown, simple, borne singly or in groups upon the substratum, 40–200 μ × 5–7 μ in diameter. Terminal cells of the conidiophores slightly contorted, bearing 3–10 brown, elongate, straight or slightly curved conidia, which are rounded at the ends, 3–15 (mostly 9–10) celled, 29.3–183.3 ×

9.1–16.5 μ (ave. 110.2 × 12.8 μ). Bulbils abundant, minute, black without, hyaline to gray within, spherical to irregular in shape, 53–276 μ (ave. 114.3 μ) in diameter.

Habitat: biogenous on *Portulaca oleracea* L.

Type locality: Watkins Glen, New York.

Type material has been deposited in the herbarium of the De-

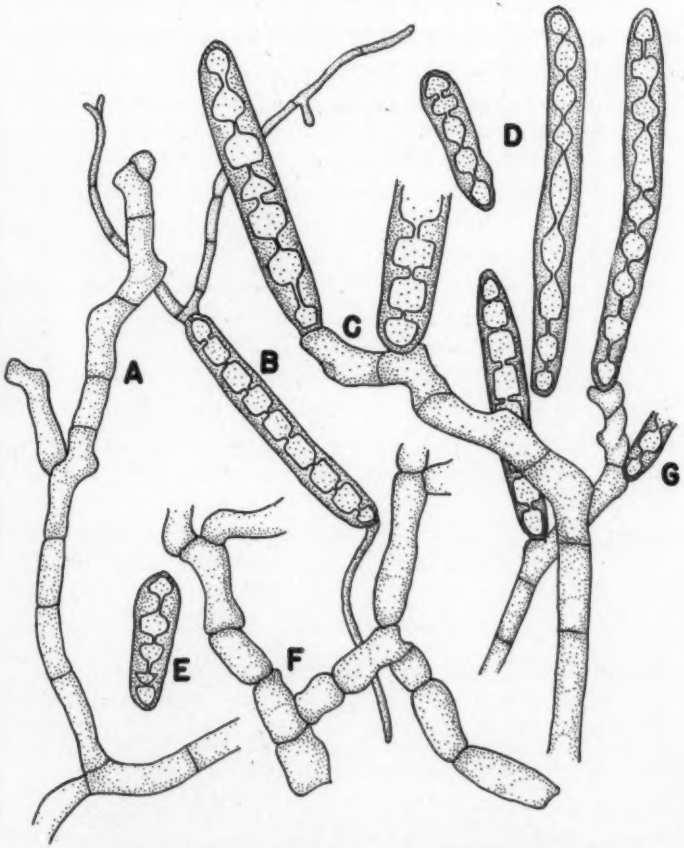

FIG. 1. *Helminthosporium portulacae*, *a*, conidiophore; *b*, germinating conidium; *c* and *g*, conidiophores showing mode of attachment of conidia; *d* and *e*, five- and nine-celled conidia; *f*, anastomosing hyphae, all drawn with the aid of a camera lucida, × 450.

partment of Plant Pathology, Cornell University, Ithaca, New York. Cultures have been sent to the American Type Culture Collection, Washington, D. C., and to the Centraalbureau voor Schimmelcultures, Baarn, Nederland.

ACKNOWLEDGMENT

The writer is indebted to Mrs. M. W. Allen of the Botany Department, Cornell University, for assistance in the preparation of the Latin diagnosis.

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HETEROTHALLIC ANTIBIOSIS IN MUCOR RACEMOSUS [1]

HUBERT A. HARRIS

During a preliminary testing of stock laboratory cultures of molds for possible antibiotic properties, a culture of a phycomycete, *Mucor racemosus* Fres., indicated antibacterial effects. It was not known whether this culture represented a plus or a minus strain of the heterothallic fungus. Inasmuch as antibiosis has been reported to occur very infrequently in the phycomycetes, the antibiotic action of *M. racemosus* was investigated further.

Wilkins and Harris (3) tested 19 phycomycetes for antibiotic action using *Escherichia coli, Staphylococcus aureus,* and *Pseudomonas pyocyanea* as test organisms. Negative results were obtained with all of these phycomycetes except for *Phytophthora erythroseptica* which exhibited antibacterial action against all three test organisms. Antibiotic tests also included heterothallic strains of nine different species of Mucoraceae. Among these were plus and minus strains of *Mucor hiemalis, M. mucedo,* and *M. sexualis.*

Waksman and Horning (2) tested six cultures of known species of *Rhizopus* against *Bacillus subtilis* with negative results.

METHODS AND MATERIALS

The strains of the fungus and the bacteria used in this investigation are as follows:

M. racemosus: plus strain, ATCC 1216a
M. racemosus: minus strain, ATCC 1216b
E. coli: ATCC 9739
S. aureus: Oxford strain H, ATCC 9144
S. lutea: stock laboratory culture, source unknown
S. marcescens: ATCC 4261.

[1] Presented in abstract form at the Meeting of the Colorado-Wyoming Academy of Science, Colorado Springs, May, 1947.

To determine possible antibiotic differences between the plus and minus strains of *M. racemosus*, the cylinder plate method of Abraham *et al.* (1), which is used with modification by many investigators of antibiotics, afforded a means for obtaining comparative data.

Plus and minus strains of the fungus were cultivated in 125 ml. Erlenmeyer flasks containing 50 ml. of Sabouraud's broth autoclaved at 15 pounds pressure for 20 minutes. The flasks were inoculated with a mycelial transfer of the fungus grown on a Sabouraud agar plate for three days at room temperature (23–27° C.). The inoculated flasks were incubated at room temperature for 10–12 days.

The contents of the flasks were thoroughly shaken and the culture liquid filtered twice through No. 1 Whatman filter paper prior to filtration through a medium (N) Berkefeld filter. Difco Bacto-Yeast Beef Agar, autoclaved at 15 pounds pressure for 20 minutes, was dispensed (25 ml.) into sterile petri dishes with a sterile pipette. The surface of the hardened agar was flooded with a 16–20 hour culture suspension of the test bacterial organism and the excess suspension was pipetted off. The bacterial test organisms were transferred three times on successive days to 10 ml. of nutrient broth and incubated at 37° C. save *S. marcescens* which was incubated at room temperature.

The surfaces of the flooded agar plates, with the covers removed, were dried for one hour in the 37° C. incubator. Four stainless steel penicillin assay cylinders (0.8 mm. O. D., 0.6 mm. I. D., and 10.0 mm. in length) were warmed in a flame and spaced equidistantly around the circumference of the plate. Alternate cylinders were then filled, respectively, with the plus and minus strain filtrates. Five plates, containing duplicate tests of the filtrates, were made for each of the bacterial test organisms. The test plates were incubated at the same temperatures employed for cultivating the various bacteria in nutrient broth.

Measurements were made to the nearest 0.5 mm. of the outside diameters of the inhibitory zones of the test organisms by means of pointed dividers and under a magnifying lens.

To test the thermostable property of the minus strain filtrate,

the paper filtrate was autoclaved at 15 pounds pressure for 30 minutes and then cooled prior to filling the cylinders.

EXPERIMENTAL RESULTS

Antibiotic tests: The results of the effects of the plus and minus strain filtrates of *M. racemosus* on the two Gram positive bacteria, *S. lutea* and *S. aureus*, and on the two Gram negative bacteria, *E. coli* and *S. marcescens*, are shown in Table 1.

TABLE 1

ANTIBACTERIAL EFFECTS OF PLUS AND MINUS STRAINS OF *M. racemosus* (MEASURED IN MM. OF INHIBITION ZONES OF DUPLICATE TESTS)

Test Organism	Plate Number	Culture Filtrate				Minus Strain Average
		Minus Strain		Plus Strain		
S. marcescens	1	13.5	13.5	0	0	13.6
	2	14.0	14.0	0	0	
	3	13.0	13.5	0	0	
	4	14.0	13.5	0	0	
	5	13.5	13.5	0	0	
S. lutea	1	23.0	19.0	0	0	18.5
	2	15.0	17.5	0	0	
	3	20.5	17.5	0	0	
	4	17.0	a	0	0	
	5	19.5	18.0	0	0	
E. coli	Negative results					
S. aureus	Negative results					

a—no measurement due to cylinder leak.

These data show that the antibiotic activity of *M. racemosus* is restricted to the minus strain of the fungus for the organisms tested. The antibiotic action of the minus strain filtrate was somewhat greater for *S. lutea* than for *S. marcescens*, and no antibiotic effect resulted with either *E. coli* or *S. aureus*.

Thermostability tests: Determinations of the thermostability of the antibiotic principle in the minus strain filtrate by autoclaving and the results obtained with *S. marcescens* and *S. lutea* are presented in Table 2.

TABLE 2

EFFECT OF AUTOCLAVING ON THE ANTIBACTERIAL PROPERTIES OF THE MINUS STRAIN FILTRATE OF *M. racemosus* (MEASURED IN MM. OF INHIBITION ZONES OF DUPLICATE TESTS)

Test Organism	Plate Number	Culture Filtrate (Minus Strain)			
		Non-autoclaved		Autoclaved	
S. marcescens	1	11.0	11.5	11.0	12.0
	2	11.5	12.0	11.0	10.5
	3	11.5	11.5	10.5	10.5
	4	11.0	11.5	11.0	10.5
	5	10.0	10.0	11.0	11.5
	Average	11.1		10.9	
S. lutea	1	19.0	18.0	17.0	17.5
	2	17.5	18.5	18.0	18.5
	3	19.0	18.0	20.0	19.5
	4	16.5	17.0	17.5	19.0
	5	19.0	18.5	19.5	19.0
	Average	18.1		18.5	

These results show that the antibiotic principle of the minus strain filtrate is thermostable and no significant differences occurred with autoclaved or non-autoclaved filtrates. Again, a greater antibiotic effect is evident for *S. lutea* than for *S. marcescens.*

SUMMARY

The phycomycete, *Mucor racemosus*, possesses antibiotic properties for certain bacteria. The active antibiotic principle is

thermostable and is restricted to the minus strain of the heterothallic fungus for the bacteria tested.

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2. **Waksman, S. A., and E. S. Horning.** 1943. Distribution of antagonistic fungi in nature and their antibiotic action. Mycologia **35**: 47–65.
3. **Wilkins, W. H., and G. C. M. Harris.** 1942. Investigations into the production of bacteriostatic substances by fungi; preliminary examination of 100 fungal species. Brit. Jour. Exper. Pathol. **23**: 166–169.

NOTES ON SOME CERCOSPORAE OF INDIA

M. J. Thirumalachar and Charles Chupp

Species of *Cercospora* included in the present study were collected in Mysore State, South India, during 1944–46. Most of the collections were made in the vicinity of Bangalore and Nandi Hills which are situated at an altitude of 3,000 ft. and 4,850 ft. above sea level respectively. The annual rain fall is about thirty inches. The present collection comprises thirty-nine different species of which twelve are described as new. Many of the commonly known species of *Cercospora* are not included. Types of the new species are deposited in the Herb. Crypt. Ind. Orient., New Delhi, India, Cornell University Herbarium, and in Herb. I. M. I., Kew, England.

Cercospora Achyranthina sp. nov.

Maculae circulares, 0.5–2 mm. diam., centro alutaceo usque griseo, margine rubro usque badio; fructificatio amphigena, praecipue epiphylla; stromata fusca e cellulis paucis usque 25 μ in diam.; caespituli ex hyphis 2–10 divergentibus compositi; conidiophora pallide olivaceo-brunnea, apices versus pallescentia attenuataque, sparse septata, non ramosa, recta vel flexuosa, 0–5 geniculata, apice anguste subtruncata, 4–5.5 × 15–80 μ, plerumque 25–45 μ; conidia hyalina, acicularia, recta vel subcurvata, indistincte multiseptata, basi truncata, ad apicem acuta, 2.5–5 × 35–130 μ.

Leaf spots circular, 0.5–2 mm. in diam., center tan to gray, margin red to reddish brown; fruiting amphigenous but chiefly epiphyllous; stromata dark brown, a few cells to 25 μ in diam.; fascicles 2–10 divergent stalks; conidiophores pale olivaceous brown, paler and narrower toward the tip, sparingly septate, not branched, straight to bent, 0–5 geniculate, narrowly subtruncate tip, 4–5.5 × 15–80 μ, mostly 25–45 μ; conidia hyaline, acicular, straight to slightly curved, indistinctly multiseptate, base truncate, tip acute, 2.5–5 × 35–130 μ.

On leaves of *Achyranthis aspera* L., Bangalore, 20–8–1944, leg. M. J. Thirumalachar. The species is distinct from *C. Achyranthis* and *C. centrostachydis* known on the same host genus.

CERCOSPORA APII Fres. Beiträge zur Mycol. Drittes Heft, p. 9. 1863.

C. penicillata var. *Apii* Fckl., Hedwigia **2**: 132–136. 1863.

On leaves of *Apium graveolens* L., Bangalore, 12–12–1945, leg. M. J. Thirumalachar.

CERCOSPORA ASPLENI Jaap. Ann. Mycol. **14**: 43, 1916.

On leaves of *Asplenium nidus,* Bangalore, 29–7–1944, leg. M. J. Thirumalachar. The collections of the fungus were made in the botanical garden of Central College, Bangalore. The large fronds were severely blemished in some cases.

Cercospora bangalorensis sp. nov.

Maculae obscurae vel atrae, subcirculares vel irregulares, 0.5–3 mm. in diam., interdum margine dilute flavidulo cinctae; fructificatio amphigena; stromata carentia vel cellulis paucis fuscis composita; caespituli ex hyphis 2–12 divergentibus consistentes; conidiophora pallide vel perpallide fuliginea, colore uniformia, non vel e basi apicem versus conspicue attenuata, dense septata saltem ad basin, non ramosa, recta vel solum leniter curvula vel flexuosa, rare geniculata, ad apicem conica, 3–5 × 10–65 μ; conidia pallide vel perpallide olivacea, anguste obclavata, recta vel leniter curvata, 1–5-septata, basi longe obconica, apice obtuso vel subacuta, 3–4.5 × 20–60 μ.

Leaf spots dark to black, subcircular to irregular, 0.5–3 mm. in diam., sometimes with faintly yellowish margin; fruiting amphigenous; stromata lacking or composed of a few dark brown cells; fascicles of 2–12 divergent stalks; conidiophores pale to very pale fuliginous, uniform in color, none to marked attenuation from base to tip, closely septate at least near the base, not branched, straight or only slightly curved or bent, rarely geniculate, tips conical, 3–5 × 10–65 μ; conidia pale to very pale olivaceous, narrowly obclavate, straight to slightly curved, 1–5-septate, long obconical base, tips obtuse to subacute, 3–4.5 × 20–60 μ.

On the leaves of *Aristolochia indica* L., Bangalore, 20–8–1944, leg. M. J. Thirumalachar.

CERCOSPORA CALOTROPIDIS Ell. & Ev. Missouri Bot. Gard. Ann. Rept. **9**: 120. 1898.

On leaves of *Calotropis gigantea* Br., Bangalore, 13–9–1945, leg. M. J. Thirumalachar.

CERCOSPORA CANESCENS Ell. & Mart. Amer. Naturalist **16**: 1003. 1882.

Cercosporina Kikuckii Mats. & Tomoy., Ann. Phytopath. Soc. Japan **1** (6): 1. 1925.
Cercospora Vignicaulis Tehon, Mycologia **29**: 436. 1937.

On leaves of *Vigna catjang* Endl., Bangalore, 22–12–1944, leg. M. J. Thirumalachar.

CERCOSPORA CANNABIS Hara & Fukui

Cercosporina Cannabis Hara, Phytopathology of crop plants, p. 195. 1928.

On leaves of *Cannabis sativa* L., Goribidnur, Mysore, 20–12–1944, leg. Thirumalachar.

CERCOSPORA CAPPARIDIS Sacc. Nuovo Giorn. Bot. Ital. **8**: 189. 1876.

On leaves of *Capparis horrida* L., Bangalore, 2–9–1945, leg. M. J. Thirumalachar.

CERCOSPORA CARBONACEA Miles. Trans. Ill. Acad. Sci. **10**: 255. 1917.

On *Dioscorea alata* ?, Nandi Hills, 19–7–1944, leg. M. J. Thirumalachar.

Cercospora Clauseniae sp. nov.

Maculae irregulares usque angulares, 0.5–4 mm. vel in areas magnas coalescentes, primum uniformiter atrobadiae, demum centro pallide brunneo usque sordide griseo; fructificatio epiphylla; stromata subglobosa, brunnea, 20–50 μ in diam.; caespituli densi, divergentes; conidiophora pallide vel pallidissime olivaceo-brunnea, colore uniformia, latitudine irregularia, 1–5-septata, rare geniculata, non ramosa, apice obtuso, 4–5.5 × 10–65 μ; conidia hyalina usque dilute olivacea, cylindro-obclavata, interdum distincte cylindrica, recta usque leniter curvata, multiseptata, basi longe obconice truncata, apice subobtuso, 3–5.5 × 30–165 μ.

Leaf spots irregular to angular, 0.5–4 mm. or coalescing into large areas, at first uniformly dark reddish-brown, later the center becoming pale brown to dingy gray; fruiting chiefly epiphyllous; stromata subglobular, brown, 20–50 μ in diam.; fascicles dense, divergent; conidiophores pale to very pale olivaceous brown, uniform in color, irregular in width, 1–5-septate, rarely geniculate, not branched, blunt apex, 4–5.5 × 10–65 μ; conidia hyaline to

faintly olivaceous, cylindro-obclavate, occasionally distinctly cylindrical; straight to slightly curved, multiseptate, base long obconically truncate, tip subobtuse, 3–5.5 × 30–165 μ.

On the leaves of *Clausenia Willdenowii* Wight & Arn., Nandi Hills, Mysore, 18–12–1945, leg. M. J. Thirumalachar.

CERCOSPORA COCCULI Syd. Ann. Crypt. Exot. **2**: 264. 1929.

On leaves of *Cocculus villosus* DC, Bangalore, 2–9–1945, leg. M. J. Thirumalachar.

CERCOSPORA COFFEICOLA Berk & Br. Grevillea **9**: 99. 1881.

On leaves of *Coffea arabica* L., Bangalore, 5–12–1944, leg. M. J. Thirumalachar.

CERCOSPORA CONSIMILIS Syd. Ann. Mycol. **23**: 423. 1945.

On leaves of *Vernonia* sp., Koppa Road, Mysore, 2–4–1945, leg. M. J. Thirumalachar. This species should probably be considered as an *Helminthosporium*.

CERCOSPORA FUSIMACULANS Atk. Jour. Elisha Mitchell Sci. Soc. **8**: 50. 1892.

Cercospora Panici Davis, Wisc. Acad. Trans. **19**: 714. 1919.
C. Panici-milacei Sawada, Descr. Catal. Formosan F. V. Rept. **51**: 31. 1931.

On the leaves of *Panicum javanicum* Poir., Bangalore, 2–9–1945, leg. Thirumalachar.

CERCOSPORA HIBISCINA Ell. & Ev. Proc. Acad. Nat. Sci. Phila. **47**: 438. 1895.

On leaves of *Hibiscus Cannabinus* L., Bangalore, 28–12–1944, leg. Thirumalachar.

Cercospora Holarrhenae sp. nov.

Maculae atrobadiae, usque fere nigra, angulares, nervis marginatae, 2–8 mm. in diam. vel in areas magnas coalescentia; fructificatio plerumque epiphylla; stromata fusca usque atra subglobosa, 20–60 μ in diam.; caespituli intense fuliginei, apicem rotundatum versus pallescentes et angustiores, rare septati, non geniculati, recti vel undulantes, 2–4 × 10–40 μ, conidia subhyalina usque pallidissime olivacea, anguste obclavata vel interdum cylindrica, recta vel curvata, indistincte multiseptata, basi brevi obconice truncata, apice subacuto vel conico, 2–4 × 20–75 μ.

Leaf spots dark reddish brown to almost black, angular, bounded by the leaf veins, 2–8 mm. in diam. or coalescing into large areas; fruiting chiefly epiphyllous; stromata dark brown to black, subglobular, 20–60 μ in diam.; fascicles very dense, slightly divergent; conidiophores pale to very pale fuliginous, paler and more narrow toward the rounded tip, rarely septate, not branched, not geniculate, straight to undulant, 2–4 × 10–40 μ; conidia subhyaline to very pale olivaceous, narrowly obclavate, or sometimes cylindrical, straight to curved, indistinctly multiseptate, base short obconically truncate, tip subacute or conical, 2–4 × 20–75 μ.

On leaves of *Holarrhena antidysentrica* Wall., Balehonnur, Mysore, 29–4–1945, leg. M. J. Thirumalachar.

CERCOSPORA IXORAE Yamamoto. Jour. Soc. Trop. Agr. **6**: 602. 1934. Reprint: Phytopath. Lab. Taihoku Imp. Univ. Contrib. **28**: 602. 1934.

On leaves of *Ixora parviflora* Vahl., Channapatna, Mysore, 4–6–1944, Nandi Hills, 15–3–1945, leg. M. J. Thirumalachar.

CERCOSPORA JASMINICOLA Muller & Chupp. Arch. Inst. Biol. Veg. Rio de Janeiro **3**: 93. 1936.

On leaves of *Jasminum rigidum* Zenk., Bangalore, 22–8–1944, and Nandi Hills, 15–3–1945, leg. M. J. Thirumalachar.

CERCOSPORA KAKI Ell. & Ev. Jour. Mycol. **3**: 17. 1887.

On leaves of *Diospyros tupru* Buch. Ham., Bangalore, 15–8–1945, Nandi Hills, 15–3–1945, leg. M. J. Thirumalachar. The leaves of *Diospyros tupru* are often made use of for making native cigarettes. Severe infection by *Cercospora* often results in defoliation.

Cercospora Lettsomiae sp. nov.

Maculae angulares, 2 mm. in diam. usque in areas magnas coalescentes; emarginatae, fuligineae demum fere atrae, infra aliquantum pallidiores; fructificatio praecipue hypophylla; stromata nulla vel cellulis paucis flavo-brunneis composita, conidiophora fasciculata ex hyphis divergentibus 5–20 consistentia, pallide usque pallidissime olivaceo-brunnea, colore uniformia, latitudine irregularia, 0–3-septata, varie curvata vel flexuosa, rare geniculata, interdum ramosa, apice obtuso usque conico, 3–5 × 10–35 μ; conidia subhyalina, usque dilute olivacea, cylindrico-obclavata vel brevissima cylindrica, recta usque 1–9-septata, basi obconica, apice obtuso, 3–4.5 × 15–75 μ.

Leaf spots angular, 2 mm. in diam. to large coalescing areas, no distinct border, fuliginous to almost black, somewhat paler on the

lower surface; fruiting chiefly hypophyllous; stromata none or composed of few yellowish brown cells; conidiophores divergent fascicles of 5–20, pale to very pale olivaceous brown, uniform in color, irregular in width, 0–3-septate, variously curved or bent, rarely geniculate, occasionally branched, blunt to conical apex, 3–5 × 10–35 μ; conidia subhyaline to faintly olivaceous, cylindro-obclavate or shortest ones cylindrical, straight to curved, 1–9-septate, base obconical, tip obtuse, 3–4.5 × 15–75 μ.

On leaves of *Lettsomia elliptica* Wight., Bangalore, 20–12–1945, leg. M. J. Thirumalachar.

CERCOSPORA MALI Ell. & Ev. Jour. Mycol. **4**: 116. 1888.

C. Piricola Sawada, Jour. Formosan Nat. Hist. Soc. **17**: 3. 1914.
C. minima Tracy & Earle, Bull. Torrey Bot. Club **23**: 206. 1896.

On leaves of *Pyrus malus*, Bangalore, 15–8–1945, leg. M. J. Thirumalachar.

Cercospora mysorensis sp. nov.

Maculae subcirculares usque angulares, 1–4 mm. in diam., obscure brunneae, interdum linea elevata marginali et halone flavida vel aurantiaca cinctae; fructificatio amphigena: stromata carentia vel cellulis paucis compositis; caespituli nulli vel hyphis 2–10 divergentibus consistentes; conidiophora pallide vel perpallide fuliginea, colore uniformia, latitudine aliquantum irregularia, 1–7-septata, recta undulata curvatave, ramosa praecipue cum non fasciculata, rare geniculata, apice obtusa rotundata, 4–6 × 20–150 μ; conidia subhyalina usque pallide fuliginea plerumque obclavata sed interdum distincte cylindrica, recta vel curvata, 1–7-septata, basi longe obconice truncata, apice plerumque obtuso, 4–6 × 20–80 μ.

Leaf spots subcircular to angular, 1–4 mm. in diam., dull brown, sometimes surrounded by a raised line border and a yellowish to orange halo; fruiting amphigenous; stromata none or a few dark cells; non-fasciculate or fascicles of 2–10 divergent stalks; conidiophores pale to very pale fuliginous, uniform in color, somewhat irregular in width, 1–7-septate, straight to undulate or curved, branched especially when non-fasciculate, rarely geniculate, bluntly rounded tip, 4–6 × 20–150 μ; conidia subhyaline to pale fuliginous, mostly obclavate but occasionally distinctly cylindrical, straight to curved, 1–7-septate, base long, obconically truncate, tip usually obtuse, 4–6 × 20–80 μ.

On the leaves of *Pouzolzia bennettiana* Wight., Nandi Hills, 17–12–1945, leg. M. J. Thirumalachar. The wide conidiophores and conidia separate this species from others with colored conidia on

the Urticaceae. *Cercospora Pouzolziae* Syd. recorded from Transvaal, Africa, is a different species.

CERCOSPORA NERII-INDICI Yamamato. Jour. Soc. Trop. Agr. **6**: 605. 1934.

On leaves of *Nerium Oleander* L., Tirumalai, Tirupati, 10–1–1946, leg. M. J. Thirumalachar.

CERCOSPORA NYMPHAEACEA Cke. & Ell. Grevillea **6**: 89. 1898.

Cercospora exotica Ell. & Ev., Proc. Acad. Nat. Sci. Phila. **45**: 463. 1893.
C. Nelumbonis Tharp, Mycologia **9**: 111. 1917.

Cercospora Panacis sp. nov.

Maculae circulares, usque 20 mm. in diam., alutaceae, usque fuscae, generaliter margine obscuriori e linea angusta elevata delimitatae, fructificatio plerumque hypophylla; stromata fusca, subglobosa, 15–40 μ in diam.; caespituli plerumque densi, paulo compacti, conidiophora pallide vel pallidissime olivaceo-brunnea, apicem rotundatum conicum versus pallescentia et angustioria, rare geniculata, non ramosa, 2–3.5 × 10–30 μ; conidia subhyalina usque pallidissime olivacea, anguste obclavata usque linearia, recta vel leniter curvata, indistincte 1–5-septata, basi obconice truncata, apice obtuso usque conico, 2–3.5 × 15–65 μ.

Leaf spots circular, up to 20 mm. in diam., tan to medium dark brown, usually with a darker margin which is separated from the remainder of the lesion by a narrow raised line; fruiting chiefly hypophyllous, stromata dark brown, subglobular, 15–40 μ in diam.; fascicles mostly dense, fairly compact; conidiophores pale to very pale olivaceous brown, paler and narrower towards the rounded to conical tip, rarely geniculate, not branched, 2–3.5 × 10–30 μ; conidia subhyaline to pale olivaceous, narrowly obclavate to linear, straight to slightly curved, indistinct 1–5-septate, base obconically truncate, tip obtuse to conical 2–3.5 × 15–65 μ.

On leaves of *Panax fruticosa* L., Bangalore, 5–8–1944, leg. M. J. Thirumalachar.

Cercospora Paramignyae sp. nov.

Maculae circulares, 4–12 mm. in diam., centro griseae vel fere atrae e fructificationibus densis atro-punctatae, margine lato albescenti vel alutaceo cinctae, fructificatio plerumque epiphylla; stromata subatra, subcircularis, 30–100 μ in diam., caespituli densissimi, compacti; conidiophora saepe solum e cellulis peripheralibus stromatis consistentia vel interdum usque 2–4 × 10–

25 μ elongata; non septati, non geniculati, non ramosi, apice fere hyalino; conidia anguste cylindrica, subhyalina, in massa olivacea, indistincte multiseptata, recta vel valde curvata, basi longe obconice truncata, apice subacuto, 1.5–3 × 25–100 μ.

Leaf spots circular, 4–12 mm. in diam., center gray or almost black with the closely stipple-like fruiting bodies, wide, blanched or tan margin; fruiting chiefly epiphyllous; stromata almost black, subcircular, 30–100 μ in diam.; fascicles very dense, compact; conidiophores often merely peripheral cells on the stroma or occasionally elongated to 2–4 × 10–25 μ, not septate, not geniculate, not branched, tip almost hyaline; conidia narrowly cylindrical, subhyaline, in mass olivaceous, indistinctly multiseptate, straight to strongly curved, long obconically truncate base, subacute tip, 1.5–3 × 25–100 μ.

On leaves of *Paramignya* sp., Balehonnur, Mysore, 24–4–1945, leg. M. J. Thirumalachar.

Cercospora petila sp. nov.

Maculae atrobadiae usque fere atrae, subcirculares usque irregulares, 2-6 mm. in diam., interdum zona rosea circumdatae; fructificatio plerumque epiphylla; stromata subglobosa, obscura, 20–40 μ in diam., caespituli densi, non compacti; conidiophora pallide usque pallidissime olivaceo-brunnea, apicem anguste rotundatam conicamve versus pallescentia et angustioria, recta usque curvata vel undulata, rare septata, non geniculata, non ramosa, 2–3.5 × 5–25 μ; conidia subhyalina vel dilute olivacea, anguste cylindrica, interdum apicem versus leniter attenuata, recta vel curvata, indistincte 1–7-septata, basi rotundata usque subtruncata, apice obtuso usque conico, 2–3.5 × 30–75 μ.

Leaf spots dark reddish brown to almost black, subcircular to irregular, 2–6 mm. in diam., occasionally surrounded by an old-rose colored zone; fruiting chiefly epiphyllous, stromata subglobular, dark, 20–40 μ in diam.; fascicles dense, not compact; conidiophores pale to very pale olivaceous brown, paler and narrower toward the narrowly rounded or conical apex, straight to curved or undulate, rarely septate, not geniculate, not branched, 2–3.5 × 5–25 μ; conidia subhyaline to faintly olivaceous, narrowly cylindrical, sometimes slightly attenuated toward the tip, straight to curved, indistinctly 1–7-septate, base rounded to subtruncate, tip blunt to conical, 2–3.5 × 30–75 μ.

On the leaves of *Euphorbia pulcherrima* R. Graham (*Poinsettia pulcherrima* L.), Bangalore, 11–11–1945, leg. M. J. Thirumalachar.

CERCOSPORA PUNICAE P. Henn. Bot. Jahrb. von Engler **37**: 165. 1906.

On the leaves of *Punica granatum* L., Bangalore, 12–1–1946, Nandi Hills, 28–12–1945, leg. M. J. Thirumalachar.

CERCOSPORA RUBI Sacc. Nuov. Giorn. Bot. Ital. **8**: 188. 1876.

Cercospora Septorioides Ell. & Ev., Field Columb. Mus. Bot. Ser. **1**: 94. 1896.
C. garbiniana Massalongo, Atti Mem. Acad. Agr. Sci. Lett. Verona Ser. 4, **3**: 147. 1902.

On the leaves of *Rubus vulgaris* L., Kemmangundi, Mysore, 9–10–1945, leg. M. J. Thirumalachar.

Cercospora Shoreae sp. nov.

Maculae irregulares, 3–10 mm. in diam., obscure griseae, linea pallide brunnea marginatae; fructificatio amphigena, plerumque epiphylla; stromata fusca, globosa, 15–35 μ; conidiophora singulatim vel e stromate in fasciculis patentibus, ad apicem obtuse rotundatam angustatis oriunda; 0–2-septata, curvata vel undulata, non ramosa, rare geniculata, 2–4 × 15–40 μ; conidia pallide olivacea, anguste obclavata usque fere linearia, recta vel leniter curvata, indistincte 1–5-septata, basi rotundata usque subtruncata, apice obtuso usque conico, 2–4 × 15–60 μ.

Leaf spots irregular, 3–10 mm. in diam., dull gray, pale brown line margin; fruiting amphigenous but chiefly epiphyllous; stromata dark brown, globular, 15–35 μ; conidiophores arising singly from the stromata or in spreading fascicles of 2–15, pale olivaceous brown, uniform in color but slightly narrower toward the bluntly rounded tip, 0–2-septate, curved to undulate, not branched, rarely geniculate, 2–4 × 15–40 μ; conidia pale olivaceous, narrowly obclavate to almost linear, straight to slightly curved, indistinctly 1–5-septate, base rounded to subtruncate, tip blunt to conical, 2–4 × 15–60 μ.

On the leaves of *Shorea talura* Roxb., Bangalore, 6–7–1944, leg. M. J. Thirumalachar. This is a new host family for *Cercospora*.

CERCOSPORA SOJINA Hara. Nogyo Sekai, Tokyo **9**: 28. 1915.

Cercospora daizu Miura, Manchurian R. R. Agr. Expt. Sta. Bull. **11**: 25. 1920.

On the leaves of *Glycine javanica* L., Bangalore, 15–8–1945, leg. Thirumalachar.

CERCOSPORA SORGHI E. & E. Jour. Mycol. **3**: 15. 1887.

C. Sorghi var. *Maydis* E. & E. (Langlois Collection No. 613).

On *Amphilophis pertusa* Stapf. (*Andropogon pertusus*), Bangalore, 22–12–1944, leg. M. J. Thirumalachar.

CERCOSPORA SUBSESSILIS H. & P. Syd. Ann. Mycol. **11**: 329. 1913.

Cercoseptoria domingensis Ciferri, Ann. Mycol. **36**: 231. 1938.

On leaves of *Azadirachta indica* A. Juss.

CERCOSPORA TECTONIAE Stevens Bernice P. Bishop Mus. Bull. **19**: 155. 1925.

On *Tectona grandis* L., 30–11–1945, Bangalore, leg. M. J. Thirumalachar.

CERCOSPORA TINOSPORAE H. & P. Syd. Ann. Mycol. **14**: 372. 1916.

On the leaves of *Tinospora cordifolia* Miers., Bangalore, 7–10–1945, leg. M. J. Thirumalachar.

Cercospora Waltheriae sp. nov.

Maculae primum indistinctae, demum badiae, angulares, 0.5–5 mm. in diam. vel coalescentes et partem grandem laminae occupantes, interdum aridae dehiscentes; fructificatio in areis oppositis in superficiem inferiorem effusa, dilute griseola usque indistincte olivacea; stromata globosa, pallide brunnea, 20–60 μ in diam.; caespituli densi; conidiophora subhyalina usque pallide olivaceo-brunnea, recta curvatave, non vel rare septata, plerumque non geniculata, non ramosa, apice obtuse rotundato, 2–3.5 × 5–25 μ, vel longioria cum conidia persistent; conidia subhyalina usque pallidissime olivacea, obclavata usque fere linearia, indistincte multiseptata, recta vel curvata, basi obconice truncata usque rotundata, apice subacuto usque obtuso, 2–4 × 35–150 μ.

Leaf spots at first indistinct, then becoming reddish brown, angular, 0.5–5 mm. in diam., or coalescing into large part of leaf surface, when dry sometimes dehiscing; fruiting or corresponding areas of the lower surface, effuse, faintly grayish to distinctly olivaceous; stromata globular, pale brown, 20–60 μ in diam.; fascicles dense; conidiophores subhyaline to very pale brown, straight to curved, not or rarely septate, mostly not geniculate, not branched, bluntly rounded tip, 2–3.5 × 5–25 μ, or when conidia are persistent, appearing much longer; conidia subhyaline to very pale olivaceous, obclavate to almost linear, indistinctly multiseptate, straight to blunt, curved, base obconically truncate to rounded, tip subacute to blunt, 2–4 × 35–150 μ.

On the leaves of *Waltheria indica* L., Bangalore, 2–9–1945, leg. M. J. Thirumalachar. The species differs from *C. Melochiae* recorded on the same host.

Cercospora Wrightiae sp. nov.

Maculae circulares, pallide brunneae usque sordide albae, margine angusto atrobadio cinctae, 0.5–4 mm. in diam.; fructificatio amphigena, stromata subglobosa fusca, 25–60 μ; caespituli densi, compacti, conidiophora pallide usque moderate olivaceo-brunnea, apicem obtuse rotundatum versus pallescentia et angustioria, sparse septata, non ramosa, rare geniculata, recta vel curvata vel flexuosa, 4–6 × 10–35 μ; conidia pallide usque moderate olivacea, obclavata, recta usque leniter curvata, 1–9-septata, basi longe obconice truncata, apice obtuso, 3–5.5 × 20–65 μ.

Leaf spots circular, pale brown to dingy white, narrow dark reddish brown margin, 0.5–4 mm. in diam.; fruiting amphigenous; stromata subglobular, dark brown, 25–60 μ; fascicles dense, fairly compact; conidiophores pale to medium olivaceous brown, paler and narrower towards the bluntly rounded tip, sparingly septate, not branched, rarely geniculate, straight to curved or bent, 4–6 × 10–35 μ; conidia pale to medium olivaceous, obclavate, straight to slightly curved, 1–9-septate, long obconically truncate base, obtuse tip, 3–5.5 × 20–65 μ.

On leaves of *Wrightia tinctora* R. Br., Bangalore, 26–12–1945. leg. M. J. Thirumalachar.

CERCOSPORA ZIZYPHI Petch. Ann. Roy. Bot. Gard. Peradeniya Parti 5.4: 306. 1909.

On the leaves of *Zizyphus oenoplia* Mill., Kemmangundi, Mysore, 8–10–1945, leg. M. J. Thirumalachar.

Grateful thanks are due to Dr. Edith K. Cash, Associate Mycologist, U.S.D.A., Beltsville, Maryland, for very kind help in rendering the diagnosis of the new species into Latin. The senior author is deeply indebted to Dr. James G. Dickson, Professor of Plant Pathology, University of Wisconsin, for the benefit of many valuable suggestions.

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PRODUCTION OF ANTIBIOTICS BY SPECIES OF MYROTHECIUM

P. W. Brian, H. G. Hemming and E. G. Jefferys

White and Downing (**13**) have recently shown that the fungus known as *Metarrhizium glutinosum* Pope (**9**), widely used in recent years in work on microbiological decomposition of cellulose fabrics, is identical with the well established species *Myrothecium verrucaria* (Alb. & Schw.) Ditm. ex Fr. A strain of *"Metarrhizium glutinosum"* has been shown (**1**, **4**) to produce a fungistatic antibiotic, isolated in pure form and named glutinosin, and a volatile highly dermatitic substance, not as yet isolated. It was therefore considered necessary, as was indeed suggested by White and Downing, to examine strains of several species of *Myrothecium* for antibiotic activity, more particularly since strains of several species of *Metarrhizium* had shown no such activity.

ORIGIN OF CULTURES

The cultures examined can all be referred to one or other of the three species described by Preston (**11**) as *Myrothecium verrucaria* (Alb. & Schw.) Ditm. ex Fr., *Myrothecium roridum* Tode ex Fr. and *Myrothecium inundatum* Tode ex Fr. They are listed below with their accession numbers in the culture collection at Butterwick Research Laboratories, Welwyn, England.

No. 173: Myrothecium verrucaria: received as *Metarrhizium glutinosum* (*U.S.D.A. 1334.2*), originally collected from deteriorated baled cotton (**7**). *No. 372: Myrothecium verrucaria:* received from Centraalbureau voor Schimmelcultures, Baarn, Holland, as *Trichurus terrophilus* Swift & Povah, this culture is almost identical in all characters with *No. 173* and in no way corresponds with the original description of *Trichurus terrophilus* (**12**). *No. 402: Myrothecium verrucaria:* received from N. C. Preston, originally collected by G. R. Bates from washings of citrus fruits in S. Rhodesia (**11**). *No. 401: Myrothecium roridum:* received from

N. C. Preston, originally collected by him (**10**) on *Viola*. *No. 433: Myrothecium roridum:* received from F. T. Brooks, originally collected by him (**5**) on *Lupinus ornatus*. *No. 434: Myrothecium roridum:* received from F. T. Brooks, originally collected by him (**5**) on *Antirrhinum*. *No. 435: Myrothecium roridum:* received from F. T. Brooks, originally collected by him (**5**) on potato haulm. *No. 436: Myrothecium inundatum:* received from N. C. Preston. This isolate (Herb. *I.M.I. 5605*) was originally collected on *Russula adusta*.

Of these, *Myrothecium inundatum* is quite distinct in its cultural characters from the others and more particularly in its much smaller conidia (ca. 4 μ in length). The cultural appearance of *Myrothecium verrucaria* and *Myrothecium roridum* varies considerably between strains of the same species and the two species cannot be distinguished by any macroscopic culture characteristics. *M. roridum No. 435* was widely different in cultural appearance from the other strains of *M. roridum* and *M. verrucaria*, being much less vigorous in growth and producing, on Czapek-Dox agar, closely woven pink mycelium instead of the fluffy white mycelium characteristic of the other strains. The only character by which the strains of *M. verrucaria* and *M. roridum* can be distinguished, as has been noted both by White & Downing (**13**) and by Preston (**11**), is the spore shape, that of *M. verrucaria* being lemon-shaped and that of *M. roridum* cylindric.

ANTAGONISM IN AGAR CULTURE

(1) *Reversed agar culture test.* This technique has been described in detail by Jefferys (**8**). It involves growing the fungus to be examined on a suitable agar medium, in this case Czapek-Dox agar, for three days, reversing the agar and inoculating the exposed reverse agar surface with conidia of *Botrytis allii*, the germination and growth of which are then observed with a low-power microscope. Results obtained with the strains of *Myrothecium* by this method are shown in Table I.

(2) *Streak test.* In this test, previously described by Brian & Hemming (**3**), plates of an agar medium suitable for growth of both bacteria and fungi are streaked with spores of the fungi to be examined, elongated colonies being produced after 3 or 4 days' incu-

bation at 25° C. The test organisms (*Endomycopsis albicans, Staphylococcus aureus* and *Salmonella typhi*) are then streaked at right angles to the mold colony and, after a further 24 hours' incubation at 37° C., inhibition is assessed in arbitrary units. Results of this test also are shown in Table I.

TABLE I

ANTAGONISM OF STRAINS OF *Myrothecium* SPP. TO FUNGI AND BACTERIA

Culture No.	Species	Reversed Agar Test *Botrytis allii*	Streak Test		
			Endomycopsis	*Staphylococcus*	*Salmonella*
173	*verrucaria*	++	++	−	−
372	*verrucaria*	++	++	−	(+)
402	*verrucaria*	++	(+)	+	−
401	*roridum*	++	−	−	−
433	*roridum*	++	−	−	−
434	*roridum*	++	−	−	−
435	*roridum*	+	−	−	−
436	*inundatum*	−	−	++	−

++ marked inhibition; + moderate inhibition; (+) doubtful inhibition; − no inhibition.

It will be seen that all strains of *Myrothecium verrucaria* and *Myrothecium roridum* produce a substance inhibitory to *Botrytis allii,* whereas *Endomycopsis,* which has been found by experience to be a somewhat more resistant mold, is inhibited only by *Myrothecium verrucaria.* Indications of possible slight antibacterial activity are shown by two strains of *M. verrucaria.* The most significant feature of these results is undoubtedly the antifungal activity of all strains of *M. verrucaria* and *M. roridum,* and the differences between the two species are probably differences of degree only, as results presented later also indicate. *Myrothecium inundatum* is in a class by itself, showing no antifungal activity but showing a well-marked inhibition of *Staphylococcus aureus.* The antibacterial activity of this fungus will be further investigated.

FUNGISTATIC ACTIVITY OF LIQUID CULTURES

Cultures were grown on 250 ml. quantities of various media in "Glaxo" culture vessels for twenty-one days and assayed for antifungal activity at intervals. The method of assay, based on a ger-

mination test with conidia of *Botrytis allii,* has been previously described (*2*); activity is expressed in BA units per ml. The media had a common basal composition (dextrose 50.0 g., KH_2PO_4 1.0 g., $MgSO_4 \cdot 7H_2O$ 0.5 g., minor element concentrate (1) 1.0 ml., distilled water 1,000 ml.), and varied in the nitrogen source as follows, the quantity of nitrogen source added being in each case sufficient to provide nitrogen equivalent to 2.3 g. KNO_3 per liter:

Medium N.....potassium nitrate
Medium AS.....ammonium sulphate
Medium AS + malate.....as AS + 0.5 per cent malic acid
Medium A.....ammonium tartrate

The media were in each case adjusted to pH 4.5 with potassium hydroxide or hydrochloric acid.

The highest assays recorded in each medium with each culture are recorded in Table II.

TABLE II

HIGHEST ASSAYS OF FUNGISTATIC ACTIVITY RECORDED DURING 21 DAYS' INCUBATION AT 25° C.

Culture No.	Species	Highest Assay (BA units/ml.) Recorded			
		N	AS	AS+Malate	A
173	*verrucaria*	64	32	256	128
372	*verrucaria*	24	128	256	128
402	*verrucaria*	64	4	32	128
401	*roridum*	24	—	64	128
433	*roridum*	32	—	64	24
434	*roridum*	16	8	48	32
435	*roridum*	8	—	8	—
436	*inundatum*	—	—	—	—

It will be seen that all strains of *Myrothecium verrucaria* and *Myrothecium roridum* produced fungistatic culture filtrates, those of *M. verrucaria* being more active than all but one (*No. 401*) of the *M. roridum* cultures. *M. roridum No. 435* was much less active than the other cultures; this strain, as has already been mentioned, was different in gross-culture appearance from the other strains. *Myrothecium inundatum* was again found to be fungistatically inactive.

It remains to be confirmed by chemical work, now in hand, whether the fungistatic substance produced by the various strains

of *Myrothecium verrucaria* and *Myrothecium roridum* is glutinosin (**4**).

DERMATITIC ACTIVITY OF CULTURE FILTRATES

Dermatitic or vesicant activity was determined by a simple patch test on the forearms of two subjects. Small circles of filter paper were soaked in culture filtrate (in each case the filtrate from Medium AS + malate was used) and strapped on the forearm for 48 hours, when the plaster was removed and the underlying skin examined. A clear positive dermatitic response was shown to all *Myrothecium verrucaria* filtrates and to all *Myrothecium roridum* filtrates except that from *No. 435,* which has also been shown to be aberrant in other respects. The filtrate from *Myrothecium inundatum* was completely inactive. The specificity of this reaction is remarkable and, in spite of all the large scale culture work with molds carried out in recent years, the only similar record is that of certain Russian workers (**6**) with a strain of *Stachybotrys alternans* Bonord.

SUMMARY

The mold previously known as *Metarrhizium glutinosum* Pope has recently been shown to be identical with the well-established species *Myrothecium verrucaria* (Alb. & Schw.) Ditm. ex Fr. A strain of *"Metarrhizium glutinosum,"* previously shown to produce, in the course of its metabolism, two biologically active substances, one highly fungistatic (glutinosin) and the other causing severe dermatitis, has been compared with several strains of *Myrothecium verrucaria, Myrothecium roridum,* and *Myrothecium inundatum.* The strains of *Myrothecium verrucaria* and *Myrothecium roridum* behaved similarly, all producing a fungistatic substance (not yet shown to be chemically identical with glutinosin) and all except one strain of *Myrothecium roridum* producing the dermatitic substance. This result emphasizes the very close relationship between *Myrothecium roridum* and *Myrothecium verrucaria.* A single strain of *Myrothecium inundatum* examined did not produce either the fungistatic or the dermatitic substance.

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MUTUAL ANTAGONISM BETWEEN PATHOGENIC FUNGI. INHIBITION OF DIMORPHISM IN *CANDIDA ALBICANS*

OTIS F. JILLSON [1] AND WALTER J. NICKERSON [2]

(WITH 2 FIGURES)

Clinically, infection by two or more pathogenic fungi in the same person is of rare occurrence. Muskatblit (**1941**) reported six cases of double infection, and reviewed thirty-six cases from the literature. He concluded that the rarity of combined fungous infections suggests the presence of a certain degree of immunity created by the first invader, which protects the individual from a superimposed infection with another fungus. Lewis and Hopper (**1943**) reported on twenty-three cases of multiple fungous infections, of which nine (five being doubtful) were considered to have combined infections in which the species of fungi were working together. Two of these cases were combined infections of *T. rubrum* and *C. albicans*. *T. rubrum* did not appear until cultures were a month old. In a later article, Muskatblit (**1946**) presented a case of dermatophytosis, of the sole of the foot, resulting from an infection with *Trichophyton mentagrophytes* (*interdigitale*) and *T. rubrum* (*purpureum*). Both fungi were isolated and grown in culture. The clinical picture, of vesicles and good response to therapy, was that of a fungous infection caused solely by *T. mentagrophytes*—rather than the dry, scaly, infiltrated, therapeutic-resistant type of lesion caused by *T. rubrum*. In addition to his earlier conclusions, Muskatblit suggested it was possible that *T. mentagrophytes* acted as an antagonist of *T. rubrum*. Since one

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usually isolates pure cultures of *Candida albicans* from lesions produced by this fungus, Lewis and Hopper (**1943**) observed that there appears to be an inhibitory effect on the growth of other microorganisms in the presence of *C. albicans*.

Our own clinical observations certainly tended to confirm the extreme rarity of mixed fungous skin infections. To see if there were any mutually antagonistic effects observable with pathogenic fungi *in vitro*, we studied two-membered pure cultures of *Trichophyton rubrum* and *Candida albicans*. Notable effects of this method of culturing these two fungi were seen on the growth rates and coloration of *T. rubrum*, and the formation of mycelia by *C. albicans*.

MATERIALS AND METHODS

The following fungi were used:[2]

Candida albicans, No. *624*, isolated from a case with chronic paronychia with nail involvement, at the Dermatologic Clinic, Boston City Hospital.

Candida albicans, No. *732*, similar source, a second case.

Candida albicans, No. *810*, isolated from a case of Perlèche.

Trichophyton rubrum (*purpureum*), No. *706*, isolated from the finger nails in a case of tinea ungium.

Stock cultures of strains of *C. albicans* were maintained on slants of Difco-corn meal agar with an additional 0.5 per cent of Bacto-agar incorporated. *T. rubrum* was maintained on Difco-Sabouraud's agar. Stock cultures were incubated at room temperature. In some experiments media buffered to a definite pH were employed. For these, McIlwain's $NaOH$-KH_2PO_4 buffer series was used.

All pH determinations were made with a Beckman glass electrode. In one series, indicator dyes were incorporated into the medium to permit visualization of the course of pH change during growth in the single and two-membered cultures. The dyes used were from a set marketed under the name "universal indicator."

[2] The assistance of Miss Louise A. Kelley with isolation and identification of the fungi is appreciated.

GROWTHS AND COLORATION OF TRICHOPHYTON RUBRUM

When *Candida albicans* and *Trichophyton rubrum* are grown together on an agar slant in a two-membered culture, mutually antagonistic effects can be observed. The wine-red color of *T. rubrum* growing singly on corn meal agar is replaced by a yellow-brown color when growing in the presence of *C. albicans*. As we show later, this color change is a result of the acid pH of the two-membered culture. The pigment of *T. rubrum* is a pH indicator. The growth of both fungi is inhibited in the mixed culture when compared with the amount of growth obtained in single-membered cultures. Mycelial development, clearly evident in the pure culture of *C. albicans*, is completely inhibited in the two-membered culture.

It was observed that cultures of *C. albicans* reached a pH of 3.0–3.2 within twenty-four hours when grown in a liquid medium with agitation. We considered the acid-producing abilities of this yeast might be responsible for so lowering the pH in the two-membered culture that the *internal* H-ion concentration of the cells of *T. rubrum* (reflecting the hydrogen ion concentration of their environment) would result in the pigment existing in a yellow-brown (acid) color.[4]

Accordingly, a series of buffered corn meal agar media was inoculated with *T. rubrum*. Good growth was obtained within the pH range employed (4.5–8.0). At pH 4.5, cultures of *T. rubrum* were yellow-brown, closely approximating the color obtained in the two-membered cultures; at pH 8.0 the coloration developed was wine-red.

Irrespective of the pH, however, the color appearing initially in cultures of *T. rubrum* on the buffered corn meal agar we employed was yellow-brown; this color then persisted or turned red in a short time depending on the pH. On Difco-Sabouraud's agar (not specially buffered) the pigment was red initially and remained so as the culture developed.

[4] Experiments *in vitro* on the pigment extracted from *T. rubrum* (and other dermatophytes) have shown it to exhibit a red color in alkaline ranges and a yellow-brown color in acid ranges. It has been suggested (Nickerson 1947) that one pigment (existing in a state determined by the intracellular physico-chemical environment) would suffice to account for the known facts relating to coloration of dermatophytes in culture.

FIG. 1. *Candida albicans* and *Trichophyton rubrum*.

Inhibition of growth of *T. rubrum* in the two-membered culture is also evident (FIG. 1). This effect of the presence of *C. albicans* may have resulted from the diffusion of metabolic products through the medium (the two organisms were not perceptibly in direct contact with each other). To examine this hypothesis *C. albicans* was grown in pure culture in a liquid medium [5] with aeration for

[5] Composition: 2.0 g. glucose, 1.0 g. glycine, 0.1 g. Difco-yeast extract, and 100 ml. distilled water.

twenty-four hours; a very heavy growth was obtained. A culture was then passed through a Corning fritted glass filter (porosity UF) to obtain a sterile filtrate which was distributed in corn meal agar media (cooled to 40° C.) to give a final concentration of 2.0 per cent filtrate by volume. One set of such tubes was autoclaved; a second set was not heated. The tubes were inoculated with *T. rubrum* and incubated at 25° C. The unheated filtrate in 2.0 per cent concentration showed considerable inhibitory effect on the growth of *T. rubrum*, whereas the heated series and controls were practically indistinguishable.

DIMORPHISM OF CANDIDA ALBICANS

One of the most interesting aspects of morphogenesis in the fungi is the conversion of cells from the single-celled, budding, yeast-like form of growth to the filamentous, mycelial pattern. This transition, and the reverse thereof, is a not uncommon phenomenon, occurring among widely diverse members of the fungi. To mention a few examples: (1) *Blastomyces dermatitidis, B. braziliensis,* and *Histoplasma capsulatum* are yeast-like at 37° C., mycelial at 25° C.; (2) *Candida albicans* is usually yeast-like during the first stages of growth on agar media, but may, under some conditions, later develop pseudomycelial and mycelial elements which may terminate with large chlamydospores; (3) species of *Mucor,* kept submerged in liquid media by agitation, exhibit a single-celled, yeast-like form of growth and may carry out an alcoholic fermentation of carbohydrates. This list could be greatly expanded. There have been several experimental investigations on methods for obtaining a yeast (Y) to mycelial (M) conversion ($Y \rightarrow M$) in culture, but the cellular mechanisms involved in such conversions have received comparatively little attention.

Variability in the yeasts producing mycelia (the *Mycotoruloideae*) is well recognized; see Wickerham and Rettger (**1939**) and Diddens and Lodder (**1942**) for extensive discussions. Whether or not a given isolate of one of these yeasts will develop true mycelia in addition to pseudomycelia is apparently unpredictable, being the result of the interaction of several known, and probably several unrecognized, variables. We have not concerned

ourselves particularly with this aspect of the problem and have restricted our attention to three strains of *Candida albicans* that readily formed mycelia on corn meal agar [6] slants (many isolates apparently require more anaerobic conditions).

Our working hypothesis with regard to the suppression of mycelium formation by *C. albicans* observed in the two-membered cultures was that metabolic product(s) of *T. rubrum* diffused through the agar medium and inhibited the development of dimorphism in *Candida*. To examine this hypothesis, sterile filtrates were prepared (as mentioned previously) from cultures of

TABLE 1

EFFECT OF ADDITION OF STERILE FILTRATES FROM *T. rubrum* BROTH CULTURES ON THE $Y \rightarrow M$ CONVERSION IN *C. albicans*

Concentration of Filtrate (Volumes Per cent)	Time of Appearance of $Y \rightarrow M$ (Days)	Rate of Mycelial Growth (Control = 1)
0	4	1
0.5	6	1
1.0	10	<1
2.0	>30	—
2.0 (heated)	9	>1
5.0 (heated)	>30	—

T. rubrum grown for two weeks at 25° C. in 125 ml. Erlenmeyer flasks containing 50 ml. of Difco-malt extract broth (initial pH 5.5). Varying quantities of such a filtrate were added to tubes of sterile corn meal agar cooled almost to solidifying (ca. 40° C.); tubes were shaken briefly after the addition of liquid and then slanted. Amounts of filtrate added were such as to give final concentrations of 0.5, 1.0, 2.0, and 5.0 per cent filtrate by volume. Identical series were prepared which were autoclaved (15 lbs. for 20 mins.)

[6] Considerable variation among lots of corn meal agar (both Difco and home made media) was observed; an attempt was made to analyze the basis of these differences. High carbohydrate content of the medium has been implicated by many authors as a reason for persistence of *C. albicans* in the yeast phase. While there may be some basis for this opinion, we have prepared different lots of Difco media with approximately identical concentrations of glucose (0.2 per cent by analysis) and observed excellent mycelial development with one lot and none with another. Additions of auxin (indole-3-acetic acid) ranging from 0.001 to 10.0 mg. per liter final concentration to a corn meal agar medium (Difco) prepared from a batch that did not support $Y \rightarrow M$ failed to induce the conversion to *M*. This was the case for a series held at 20° C. and for another held at 25° C.

after addition of the filtrates to the tubes. Tubes in the two series were inoculated with *C. albicans* and incubated at 25° C.

As seen in Table 1, the filtrate from *T. rubrum* possessed marked ability to inhibit mycelial development by *C. albicans*. This property was altered to a considerable extent by heating. The unheated filtrate had very little (and the heated filtrate even less) inhibitory effect on the growth of *C. albicans* as a yeast (FIG. 2). The heated filtrate in 2.0 per cent concentration retarded mycelial development for five days. Once initiated, development of mycelium was relatively more rapid in cultures with heated filtrate (up to 2.0 per cent concentration) than in the controls; chlamydospores were produced as they were in the controls. Unheated filtrate in a concentration as low as 2.0 per cent completely prevented the $Y \rightarrow M$

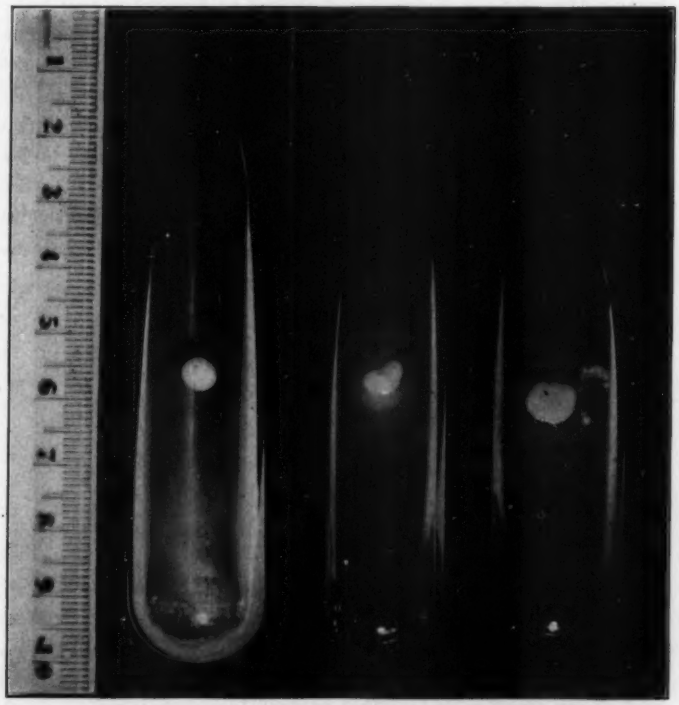

FIG. 2. *Candida albicans* and *Trichophyton rubrum*.

conversion (cultures examined for a thirty day period); only yeast cells were visible microscopically in such cultures; the *Y* cells were apparently normal and exhibited dimorphism when transferred to plain corn meal agar media within the usual time of three to four days.

PRELIMINARY FRACTIONATION OF T. RUBRUM FILTRATES

A *T. rubrum* filtrate (prepared as described above) was treated with charcoal (0.2 g. Norite/100 ml. filtrate) at 50° C. with vigorous stirring. The charcoal was removed by filtration through Whatman paper (no. 50) and the filtrate sterilized by passing through a fritted glass filter. This sterile filtrate was tested for activity. The residue from the Norite treatment was eluted with water containing 1.0 per cent acid alcohol by volume. This mixture was filtered and sterilized as just described and the sterile eluate tested for activity. The filtrate from another identical culture of *T. rubrum* was concentrated *in vacuo* to a thick syrup. To this twenty volumes of acetone were added; the acetone-soluble fraction was separated by filtration, evaporated to dryness under reduced pressure, and dissolved in a volume of water equal to the original filtrate. The acetone insoluble material was dissolved in a similar volume of water. Each of these aqueous solutions was sterilized by filtration and tested for activity.

TABLE 2

PRELIMINARY FRACTIONATION OF *T. rubrum* FILTRATE FOR *Y*→*M* INHIBITORY ACTIVITY

Fraction (All 2 Per cent by Vol.)		Time of Appearance of *Y*→*M* (Days)
Control		5
Norite adsorbed	heated	10
	unheated	10
Not adsorbed	heated	5
	unheated	7
Acetone soluble	heated	7
	unheated	7
Acetone insoluble	heated	5
	unheated	7

It is evident from Table 2 that much of the mycelium-inhibiting activity of the *T. rubrum* filtrate has been adsorbed by the Norite, and that it can be eluted from the Norite (the eluting agent tested by it-

self was without effect). The Norite-adsorbed fraction is heat stable. Heat stable inhibitory activity is also found in the acetone-soluble fraction. The heat labile inhibitory activity appears to reside in the acetone-insoluble and Norite-non-adsorbed fractions. The inhibitory action of *T. rubrum* on the *Y*→*M* conversion in *C. albicans* thus appears to be due to two diffusible metabolic products: (1) soluble in water and acetone, heat stable, and adsorbed by Norite from which it can be eluted by dilute aqueous-acid alcohol; (2) water soluble, acetone insoluble, heat labile, and not adsorbed by Norite.

EFFECT OF pH AND NH_4 CONCENTRATION ON DIMORPHISM OF CANDIDA

It is well known that cultures of dermatophytes become alkaline; Goddard (**1934**) showed this to be a result of the production of ammonia—probably by the oxidative deamination of amino acids. We examined the possibility that pH or the ammonium ion might be involved in the suppression of dimorphism in *C. albicans*. Inoculations onto slants of buffered corn meal agar (prepared as described previously) resulted in mycelial production at all H-ion concentrations (4.5–8.0) within four days; at pH 4.5 mycelial formation was evident in three days.

TABLE 3

EFFECT OF ADDITION OF NH_4Cl ON *Y*→*M* CONVERSION IN *C. albicans*

Concentration of NH_4Cl (Per cent w/v)	Time of Appearance of *Y*→*M* (Days)
0.0	4
3.0 (unheated)	>15
3.0 (heated)	>15
1.0 (heated)	>15

Ammonium chloride, added to corn meal agar to give final concentrations of 0.05, 0.1, 0.5, and 1.0 per cent NH_4Cl, resulted in rather erratic appearance of mycelial growth; with most cultures the appearance of *M* was delayed, but in some tubes with the lower concentrations of NH_4Cl the *Y*→*M* conversion was as rapid as in the controls, and in one experiment the cultures with NH_4Cl (all concentrations) actually appeared a day earlier in the *M* form than did the controls. An additional series with higher concentra-

tions of NH_4Cl was studied (TABLE 3). Consistent inhibition of $Y \rightarrow M$ was obtained with 3 per cent NH_4Cl in the medium.

Ammonia production by *T. rubrum* is very small during the first seven days growth in broth cultures, but increases rapidly thereafter. The change in pH in such cultures, due chiefly to the production of ammonia, is likewise small during the first week of incubation. In the two-membered cultures (FIG. 1) it is clearly evident that the active metabolic products of *T. rubrum* must be produced in sufficient quantity by the fourth or fifth day of incubation (judging from the time of $Y \rightarrow M$ conversion in pure culture) to prevent $Y \rightarrow M$ in *C. albicans*. The total ammonia produced (expressed as NH_4Cl) by *T. rubrum* in broth cultures after thirty days incubation is certainly less than 0.1 per cent. Since our results with such low concentrations of NH_4Cl were inconclusive and, in view of the small quantity of ammonia produced by *T. rubrum* during early stages of growth, it must be concluded that the dimorphism inhibitory activity of *T. rubrum* filtrates cannot be attributed to the NH_4^+ ion or to a change in the pH of the medium.

GENERAL ASPECTS OF $Y \rightarrow M$ DIMORPHISM

Several examples of fungi capable of existing in dimorphic states, as *Y* or *M*, were presented earlier. The phenomenon of dimorphism among microorganisms is quite widespread; thus the prominence of the feature among pathogenic forms may be more apparent than real. In addition to examples given previously, dimorphism is seen among the Protozoa in *Leishmania* and *Histomonas* which are amebic in tissues and flagellate in culture.[7] The smuts are yeasts in culture and mycelial in their plant hosts.

Among the bacteria the production of a filamentous type of growth can be brought about in a variety of ways (TABLES 4, 5, and 6); Hinshelwood (**1946**) has discussed the problems at length from a physico-chemical point of view and Lea (**1947**) from the

[7] We are indebted to Professor L. R. Cleveland for drawing our attention to this analogous situation among the pathogenic protozoa. It is interesting to see that here too the dimorphism expressed could serve as a basis for classification of the organism in one or another of two large groups—exactly as might be done, in some instances, for the fungi on the basis of a *Y* or an *M* form.

TABLE 4

CONDITIONS FAVORING $Y \rightarrow M$ OR $B \rightarrow F$ CONVERSIONS

Condition	Organism	Remarks	Author
1. Nutritional:			
a. Di- or polysaccharide carbon source	*C. albicans*		4, 18
b. NH_4^+ nitrogen source	*B. lactis aerogenes*		9, 10
c. Transfer from NH_4^+ to NO_3^- nitrogen source	*E. coli* and *B. lactis aerogenes*		9, 10
2. Specific substances:			
a. Methyl violet	*E. typhosum*		1
b. Proflavine	*B. lactis aerogenes*		9, 10
c. *m*-Cresol	*B. lactis aerogenes*		9, 10
d. Penicillin	Many Gram − and Gram + bacteria	Effect obtained at 10–30-fold dilutions of bacteriostatic concentrations	5
e. Filtrate from grown cultures of *B. lactis aerogenes*	*B. lactis aerogenes*	Shortens lag phase; L-factor favored more than D-factor	9, 10
3. Physical agents:			
a. Ionizing radiations	*E. coli*, etc.	Usually permanent effect	3, 7, 12, 14, 15, 28
b. Elevated temperature (40° C.)	*B. lactis aerogenes*	Cells normal at 30° C. even in presence of proflavine or *m*-cresol	10, 11
c. Lowered temperature (<ca. 30° C.)	*Blastomyces dermatitidis* and *B. braziliensis*	Thermal dimorphism	16, 25
d. Osmotic pressure (M/20 NaCl)	*B. lactis aerogenes*	Enhances the proflavine or *m*-cresol effect	10, 11

TABLE 5

CONDITIONS INHIBITING $Y \rightarrow M$ OR $B \rightarrow F$ CONVERSIONS

Condition	Organism	Remarks	Author
1. Nutritional:			
a. Amino-acid-nitrogen source	*B. lactis aerogenes*	Division keeps pace with elongation; only "normal" cells seen	9, 10
b. Dextrose-carbon source	*C. albicans*		4, 8, 18, 27
c. Adequate aeration	*C. albicans*	Some strains	4, 8, 27
2. Specific substances:			
a. *T. rubrum* filtrate	*C. albicans*	Two fractions (see text)	This paper
3. Physical agents:			
a. Osmotic pressure	*B. lactis aerogenes*	Except in critical range of M/20 NaCl, osmotic pressures inhibitory	10, 11
b. Elevated temperature (34–37° C.)	*Blastomyces dermatitidis* *B. braziliensis*	Thermal dimorphism, apparently independent of nutritional level	16, 25

recent work with ionizing radiations. Hinshelwood cites conditions favoring the formation of abnormally long cells as (a) presence of certain drugs which inhibit division without inhibiting growth to the same extent—for example, Ainley-Walker and Murray (**1904**) obtained filaments by growing *Bact. typhosum* in the presence of methyl violet and other dyes; and (b) transfer of the cells to an unaccustomed medium to which the growth and division functions adapt themselves at different rates.

Hinshelwood and Lodge (**1944**) observed very long filaments with *Bact. lactis aerogenes* on transfer from bouillon to a glucose-inorganic salts-ammonium sulphate medium in which the glucose concentration was low (0.19 per cent). This we may designate as a bacterium to filament conversion ($B \rightarrow F$) in analogy to $Y \rightarrow M$ with *Candida*. With a higher glucose concentration (3.85 per cent), the same medium supported a population of only normal-sized cells. They viewed this as a lack of adaptation of the cells on the transfer from bouillon to the low glucose medium; serial subculture in either glucose medium was accompanied by a gradual disappearance of filamentous cells on inoculation into the dilute glucose medium. Himshelwood (**1946**) developed the hypothesis that two separate factors favor elongation (L factor) and division (D factor) respectively, with the former diffusible into the medium. Amino acid media, in contrast to ammonium sulphate media, favor the production of factor D, and $B \rightarrow F$ was never observed in a glucose-asparagine medium. A filtrate from cultures of *Bact. lactis aerogenes* hastened the onset of growth, shortening the lag period, when added to culture media; in some instances cell division was not stimulated correspondingly thus favoring the conversion of $B \rightarrow F$.

From a genetical viewpoint it is clear that the dimorphic phenomena in *C. albicans*, and in some of the other examples discussed (TABLE 4), cannot be considered as mutations. The morphogenetic conversions are not stable and will revert to the original form on the return of the elongated cells to a suitable environment; furthermore, it is apparent in many cases that a high proportion of a population has undergone the $Y \rightarrow M$ or $B \rightarrow F$ conversion. Figure 2 shows the occurrence of $Y \rightarrow M$ from the entire periphery of

the *Y* colony. In other instances it appears probable that *B*→*F* may result from a mutation as judged by the permanence of *F*.

The mutation-producing action of X-irradiation is well known since the first demonstration by Muller (**1927**) on *Drosophila*. By such techniques Haberman and Ellsworth (**1941**) obtained elongated cocci from *Staphylococcus aureus*. Kempster (**1917**) early observed that some cultures of bacteria that had been X-irradiated might fail to divide but were not dead and would continue to elongate into filamentous elements. Such elongated forms have been subsequently studied by Lea et al. (**1937**), Witkin (**1946**) and others. Eisenstark and Clark (**1947**) examined greatly elongated cells of *Escherichia coli* (X-ray induced) in the electron microscope; they found definite "breaks" in the cell spaced periodically along its length, possibly at the sites where the cell normally would have divided. They advanced the hypothesis that an "enzyme responsible for pinching off the cell wall in normal bacterial fission has been destroyed by rays while other enzyme systems continue to function."

From the recent investigations of Knaysi (**1944**) and Robinow (**1945**) it is clear that localized events inside the cell precede the fission of the cell and probably exert the causative effect; neither worker found any indication of cell division by simple constriction.

If we consider the division of cells to be controlled by a single enzyme or enzyme complex (D factor of Hinshelwood) then it

TABLE 6

THEORIES ADVANCED TO EXPLAIN THE *B*→*F* AND *Y*→*M* PHENOMENA

Author	Theory
1. Linossier & Roux 1890	"The complexity of form of the thrush parasite (*C. albicans*) is proportional to the molecular weight of the food elements of the medium (8)."
2. Henrici 1930	Conditions favoring rapid growth of *C. albicans* cause *Y* to predominate; less favorable conditions encourage appearance of *M* form.
3. Hinshelwood 1946	Cell Division (D) and Elongation (L) under control of two independent factors that can get out of balance, as judged by statistical distribution of cell sizes, from various causes.
4. Eisenstark & Clark 1947	A "pinching off" enzyme responsible for cell division is inactivated (by X-rays) while other enzymatic activities are unhampered. (Compatible with the "D factor" of Hinshelwood.)

would follow that substances acting as inhibitors of this system would occasion a lag in division and possibly the appearance of $B \rightarrow F$ and $Y \rightarrow M$. Since inhibition of such an enzyme system would probably not result in rapid death of the cell, we would find an explanation for the reversion of $F \rightarrow B$ and $M \rightarrow Y$ when the inhibitory agent is removed and the cell restored to an environment supporting normal growth. Should the "pinching off" enzyme system be permanently inactivated (and the genic method for its regeneration [8] abolished) the conversion of $B \rightarrow F$ and $Y \rightarrow M$ would assume a permanent character.

DISCUSSION

The data presented on the *in vitro* effects of association of *Trichophyton rubrum* and *Candida albicans* in two-membered pure cultures indicate the latter exerts a fungistatic action on the growth of *T. rubrum*. Filtrates from liquid cultures of *C. albicans* had a similar action, the activity being destroyed on autoclaving. These findings may, in part, explain the clinical findings that *C. albicans* is usually isolated from lesions as a pure culture. We are not in a position at present to say anything about the pathogenicity of either species after treatment with metabolic products of the other.

Filtrates from broth cultures of *T. rubrum* did not noticeably affect the growth of *C. albicans* as a yeast but completely suppressed the appearance of the normal phenomenon of yeast to mycelium conversion in the three strains studied. In the general aspects of the morphogenetic problem presented as $Y \rightarrow M$ or $B \rightarrow F$ specific instances of interference with morphogenetic pattern are seen in the action of agents promoting cell elongation, possibly through interference with cell division. If this be accomplished, as the accumulated evidence appears to indicate, by the inhibition or total

[8] McIlwain (**1946**), in a paper of far-reaching significance, has calculated that some enzymes may exist as only one or a few units in a microbial cell, suggesting that in microorganisms some enzymes may be identical with the respective genes—the enzyme having a dual hereditary and enzymatic function. It is doubtful that the hypothetical "pinching-off" enzyme would be involved in any of the more rapid cell reactions (respiration, protoplasmic synthesis, cell wall elaboration, etc.) that proceed during its inactivation. Therefore, it possibly has a low turnover number and might belong to the group McIlwain regards as existing as one or at most a few units in a cell.

inactivation of a unit enzyme system responsible for cell division, the equivalent action of diverse chemical and physical agents becomes more understandable. Non-appearance of the *M* stage in *C. albicans* (i.e., maintenance of the population in the *Y* state) in a strain where $Y \rightarrow M$ is a normal occurrence in culture is more difficult of interpretation. In general, an environment supporting good growth causes the population to appear only as the *Y* or *B* form (see TABLE 5); to use Hinshelwood's interpretation, the growth and division factors in the cells of the population are well balanced. Since the *T. rubrum* filtrates appeared to be without effect on the growth rate of the *Y* form of *C. albicans* in a good nutrient medium, one might conjecture that the normal occurrence of $Y \rightarrow M$ in *C. albicans* cultures after the third or fourth day is stimulated or occasioned by metabolic products of *C. albicans* and this effect is specifically neutralized by the active *T. rubrum* fractions.

SUMMARY

By means of two-membered pure cultures the pathogenic fungi, *Trichophyton rubrum* and *Candida albicans*, have been shown to exert antagonistic effects on one another *in vitro*. The effects observed were (1) inhibition of growth (marked inhibition of *T. rubrum* by a heat-labile metabolic product of *Candida*), (2) the production of a yellow-brown color by *T. rubrum* in the two-membered cultures as a result of acid production by *Candida* (the pigment of *T. rubrum* is a pH indicator), (3) complete inhibition of mycelial production by *C. albicans*.

The latter phenomenon was studied in some detail. Two metabolic products of *T. rubrum* were shown to inhibit the yeast to mycelial conversion (designated as $Y \rightarrow M$). These products were: (1) soluble in water and in acetone, heat stable, adsorbed by Norite and eluted from it by dilute aqueous acid alcohol, (2) soluble in water, insoluble in acetone, heat labile, not adsorbed by Norite.

Conversion of $Y \rightarrow M$ was independent of pH (4.5–8.0), but was inhibited regularly by high concentrations of NH_4Cl (3 per cent). It was concluded the active fractions of *T. rubrum* filtrates were not the H^+ or NH_4^+ ions.

The general phenomenon of inhibition of cell division without simultaneous inhibition of growth (resulting in the production of elongated cells) is discussed in some detail. The possibility of inhibition or inactivation of a single enzyme complex responsible for cell division is briefly considered.

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EXPLANATION OF FIGURES

FIG. 1. Two-membered pure culture (right) of *Candida albicans* (upper) and *Trichophyton rubrum* (lower). Single-membered cultures of *C. albicans* (left) and *T. rubrum* (center). Note the complete suppression of mycelial development in *C. albicans* in the 2-membered culture. The coloration of *T. rubrum* in the same culture was a burnt orange, while in the single culture (center) it was characteristic wine red. Note differences in size of the two growths.

FIG. 2. Effect of *T. rubrum* filtrates on $Y \rightarrow M$ in *Candida albicans*. 14 day cultures of *C. albicans* on corn meal agar; left, 2 per cent unheated filtrate from broth culture of *T. rubrum;* center, control on plain corn meal agar; right, 2 per cent autoclaved filtrate of *T. rubrum* culture. Complete suppression of mycelia at left; absence of inhibitory effect at right. The authors thank Mr. White for assistance with the photographic work.

NOTES AND BRIEF ARTICLES

A Cellulose-degrading Gliocladium

A *Gliocladium* (identified by K. B. Raper (1) as a member of the *roseum* series) shows considerable ability to degrade cellulose. This organism was isolated from a sedge peat marketed commercially as "Michigan Peat." As far as it has been possible to ascertain, this character has not been previously reported for members of this series.

Using unsized cotton strips and testing for loss of tensile strength with a Scott breaking strength tester, this organism in one month produced degradation to such an extent as to show a loss in tensile strength of 64.7 per cent. Similar results were obtained recently by White (2) at the Biological Laboratories of the Quartermaster Depot in Philadelphia, using another strain (Fla. B–34) of the *G. roseum* series.

This percentage loss was determined by using 3.7 ounce cotton strips 6 inches by $1\frac{3}{16}$ inches. The strips were leached in several changes of distilled water, sterilized by autoclaving and oven dried at 65° C. These strips were then placed in French square bottles containing 35 ml. of Czapek's Mineral Agar composed as follows: $NaNO_3$ 2.0 grams, K_2HPO_4 1.0 gram, $MgSO_4$ 0.5 gram, KCl 0.5 gram, $FeSO_4$ 0.01 gram, agar 15.0 grams, water 1,000 ml. The inoculation was made by pipetting 1 ml. of a heavy spore suspension onto the surface of the strip. Twelve bottles were inoculated and incubated at 30° C. In groups of 4 bottles each, these were harvested at the end of periods of 1, 2 and 4 weeks. A series of 4 uninoculated strips was subjected to all the processing and incubation given the test strips. These strips were used as a control and the standard of tensile strength. After the strips were removed from the bottles they were immersed in formalin for 3 minutes and then washed in running water, oven dried at 65° C. and stored in a desiccator until time for testing. The formalin treatment used here to preclude the possibility of any further growth has been found by Hutchinson (3) to have no appreciable effect on the

tensile strength of such cotton strips. Prior to testing, the strips were hung for 24 hours in a chamber which was maintained at a constant temperature of 21° C. and a relative humidity of 65 per cent. The strips were tested in this chamber on a Scott Break Testing Machine. The average breaking strength for each group was compared with the breaking strength of the average of the control strips. The following table gives the result:

Duration of Growth	Breaking Strength in lbs.				Average, lbs.	Loss of Tensile Strength, Per Cent
	Sample 1	2	3	4		
One week	34	28	32	29	30.7	26.9%
Two weeks	31	24	19	24	24.5	41.7%
Four weeks	14	14	16	15	14.75	64.7%
Control (four weeks)	42	40	45	41	42.0	00.0%

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NOTICES

JOHN LEWIS SHELDON. The death of John Lewis Sheldon, professor of bacteriology and later of botany at West Virginia University, 1903–1919, occurred at Morgantown, W. Virginia, on Jan. 15, 1947, in his 82nd year. Dr. Sheldon took his doctorate at the University of Nebraska under the guidance of Dr. Charles Bessey, that most eminent of American botanical teachers, and was one of the group of Bessey's early students who have had a marked influence on American botany.

During his active years at West Virginia Sheldon carried out a series of phytopathological studies, giving particular attention to the taxonomy and life histories of the several fungi concerned. His published papers include reports on fungi associated with apple and guava diseases, on various anthracnose fungi, and on a number of rusts. Among fungi named by him were the ubiquitous *Fusarium*

moniliforme and *Illosporium malifoliorum*, the latter associated with an apple leaf spot.

Following his withdrawal from the University he continued his interest in the botany of his adopted State, studying especially mosses, fungi, and lichens. His studies of the latter culminated in an extensive paper on the lichens of West Virginia (Castanea **4**: 75–136. 1939), his last published paper. His extensive botanical collections presumably will be ultimately incorporated into the herbarium of West Virginia University.

More extended accounts of the life and work of Professor Sheldon appeared in Castanea (**4**: 69. 1939) and in Science (**105**: 541. 1947). The first of these includes a portrait and a bibliography.—JOHN A. STEVENSON.

PAUL MARSHALL REA. News has been received that Paul Marshall Rea, former director of the Santa Barbara Museum of Natural History, died suddenly at his home, 436 E. Padre Street, Santa Barbara, California, January 15, 1948. As a member of the Mycological Society he was active in the study of the fleshy fungi of the region around Santa Barbara, and he built up an herbarium of more than 1,600 specimens. He published on a number of his discoveries, and at the time of his death was occupied with a study of *Lysurus*. His greatest fame, however, was in museum circles where he was nationally known. He was a former president of the American Association of Natural History Museums, and author of several books on this type of museum and its relation to the community.

He was born in Potuit, Massachusetts, February 13, 1878, was a graduate of Williams College and did graduate work at Columbia University. He taught at the University of South Carolina before going to Cleveland as the first director of the Cleveland Museum of Natural History. In 1931 he resigned from the Cleveland Museum and went to Santa Barbara and became the director of the natural history museum there. He retired in 1941. He is survived by Mrs. Marion Goddard Rea; a daughter, Dorothy Helen Rea of Berkeley, California; and a son, John Morse Rea of Garden City, L. I., and three grandchildren.—ALEXANDER H. SMITH.

HENRY C. BEARDSLEE. News reached me a short time ago of the death of Mr. Beardslee, one of our well known members and for many years a critical student of the fleshy fungi. A more detailed account of his life and work will appear in a forthcoming issue of Mycologia.—ALEXANDER H. SMITH.

WILLIAM H. LONG. The death of William H. Long, retired forest pathologist of the Bureau of Plant Industry, and for some years a member of the Mycological Society, is noted. Dr. Long passed away at his home in Albuquerque, New Mexico, where he had lived since retirement in 1937, on December 10, 1947. A more extensive account of his life and work will be presented later.—JOHN A. STEVENSON.

CORRECTIONS: In the article by Roberts in the March–April issue the figures were interchanged. Fig. 1 should read *N. profusa* and Fig. 2 *N. ramosa*.—A. H. S.

In the recently published directory of the Society H. R. Rosen's address was erroneously given as Rahway, N. J. It is Univ. of Arkansas, Fayetteville, Ark.—A. H. S.

WANTED—Mycologia, vols. 31, 32, 36, 37

The stock of volumes 31, 32, 36 and 37 of MYCOLOGIA is running low, and of certain numbers is exhausted. To fill future orders for back sets it is desirable to build up the supply. Until enough are again on hand, these volumes or the needed parts will be purchased at the rate of $5.00 a volume. Those who do not need and are willing to sell these volumes will assist the journal by reporting that fact to the Managing Editor, Mycologia, New York Botanical Garden, New York 58, N. Y.—DONALD P. ROGERS.